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CHEMICAL CONTROL OF CEREAL RUSTS. IV. THE INFLUENCE OF NICKEL COMPOUNDS ON WHEAT, OAT, AND SUNFLOWER RUSTS IN THE GREENHOUSE[1]

F. R. Forsyth and B. Peturson[2]

SUMMARY

Greenhouse tests have shown that leaf rust (*Puccinia rubigo-vera* (DC) Wint. f. sp. *tritici* (Eriks.) Carleton) and stem rust (*Puccinia graminis* Pers. var. *tritici* Eriks. & E. Henn.) of wheat can be controlled by rather low concentrations of nickel compounds, through both protective and eradicative action. Oat and sunflower seedlings are more sensitive than is the wheat plant to phytotoxicity caused by the nickel. Control of crown rust of oats (*Puccinia coronata* Corda f. sp. *avenae* Eriks. & E. Henn.) and of sunflower rust (*Puccinia helianthi* Schw.) would be practical only at the low concentrations of nickel required for protective action, since the concentrations effective in eradicative action on oats and sunflower seedlings were very close to the level toxic to these plants. Simulated rain removes the protective action of water-soluble nickel salts more easily than that of the nickel salt amine complex.

Keil, Frohlich, and Van Hook[3] found that certain salts of amine complexes, as well as other experimental chemicals, were very promising as protective and eradicative fungicides for the control of rye leaf rust caused by *Puccinia rubigo-vera* f. sp. *secalis* in the greenhouse. Among the complexes tested were derivations from zinc, copper, cadmium, and nickel. Of these, only the nickel analog gave satisfactory eradication of rye rust. These investigators then showed that the active eradicative constituent of the nickel salt amine complexes was the nickel portion of the molecule. In 1956 it seemed advisable to cross-compare selected experimental materials having demonstrated greenhouse activity in order to determine their field performance in the control of leaf rust of wheat. Peturson, Forsyth, and Lyon[4] reported the results of these investigations, which demonstrated the outstanding performance of several nickel salt amine complexes. During the winter of 1956-57, cooperative laboratory investigations were undertaken with the Rohm & Haas Company in an attempt to clarify the fungicidal activity of nickel. The experimental results were detailed by Keil, Frohlich, and Glassick[5] in manuscript III of the present series. The present paper (IV) reports results obtained in the Canada Department of Agriculture Research Laboratory, Winnipeg, Manitoba.

In these greenhouse tests, it seemed desirable to compare the activity of a nickel salt amine complex with the activities of the parent amine and of its constituent nickel salt alone. Following initial tests, a program was carried out to test nickel as a protective and eradicative fungicide for leaf and stem rust of wheat, crown rust of oats, and sunflower rust. Water was sprayed on wheat seedlings that had been sprayed with a nickel salt amine complex, and on seedlings that had received only soluble nickel salts, to determine how readily these chemicals were removed by simulated rain.

MATERIALS AND METHODS.—Plant material for comparison of the component parts of the nickel salt amine complex bis[*N*-(2-hydroxyethyl)dodecylmethylbenzylamine] nickel (II) chloride, hereinafter referred to as RH-1, was obtained by growing Little Club wheat seedlings in soil in 4-in. earthenware pots. When the first leaves of the seedlings were about 3 in. long they were inoculated with race 15B of *P. graminis* f. sp. *tritici*. On the 6th, 8th, and 11th days after inoculation the chemicals were sprayed on the infected leaves to test their effectiveness in eradicative action. The fungicidal sprays were applied at the rate of 8 ml per pot of 8–10 seedlings with a DeVilbiss No. 15 atomizer operated by compressed air. When half of the solution had been applied, the seedlings were turned 180° and the remainder of the solution was applied.

Marquis or Little Club seedlings of wheat were used for tests of the protective and eradicative activities of the inorganic nickel salts against stem and leaf rusts of wheat.

When a test was intended to assess protective activity, the chemicals were applied to the surface of the first seedling leaf by atomizing 8 ml of solution on the 8–10 seedlings per pot. After the leaves had dried, rust urediospores were applied by drawing the plant leaf between the thumb and forefinger. The seedlings were then incubated 24 hours in a moist chamber,

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[2] Plant Physiologist and Plant Pathologist, respectively, Canada Department of Agriculture Research Laboratory, Winnipeg, Manitoba, Canada.

[3] Keil, H. L., H. Frohlich, and J. O. Van Hook. 1958. Chemical control of cereal rusts. I. Protective and eradicative control of rye leaf rust in the greenhouse with various chemical compounds. Phytopathology 48: 652-655.

[4] Peturson, B., F. R. Forsyth, and C. B. Lyon. 1958. Chemical control of cereal rusts. II. Control of leaf rust of wheat with experimental chemicals under field conditions. Phytopathology 48: 655-657.

[5] Keil, H. L., H. Frohlich, and C. E. Glassick. 1958. Chemical control of cereal rusts. III. The influence of nickel compounds on rye leaf rust in the greenhouse. Phytopathology 48: 690-695.

TABLE 1.—*The eradicative activity of RH-1,[a] $NiCl_2 \cdot 6H_2O$, and the parent amine of RH-1 against race 15B$_1$ of P. graminis tritici on Little Club wheat seedlings*

Chemical	Nickel content ppm	Eradicative action[b] with 3 spraying times		
		On day of flecking[c]	2 days after flecking	5 days after flecking
RH-1	280	+	++	—
RH-1	140	+	++	—
$NiCl_2 \cdot 6H_2O$	40.3	+	+	—
$NiCl_2 \cdot 6H_2O$	145.3	+	++	—
$NiCl_2 \cdot 6H_2O$	403.0	+	++	—
Parent amine[d] 3040 ppm	None	—	—	—
Parent amine[d] 6080 ppm	None	—	—	—

[a] The nickel salt amine complex bis[*N*-(2-hydroxyethyl) dodecylmethylbenzylamine] nickel (II) chloride.

[b] + = fair eradicative action; ++ = excellent; — = none.

[c] At the temperatures of this experiment, flecking occurred 6 days after inoculation.

[d] *N*-(2-hydroxyethyl) dodecylmethylbenzylamine.

and the numbers of uredia on each leaf were counted 10 days later.

When the chemicals were to be compared for eradicative activity, the rust urediospores were applied by hand, as above, and the seedlings were incubated 24 hours. The chemicals were applied in the same manner as chemicals tested for protective activity, but on the day after the first outward sign of infection (flecking, about the 6th day in the greenhouse tests). The effectiveness of the chemicals as eradicative fungicides was determined by noting whether development of the uredia was arrested.

Tests with oats and sunflowers were done in the same manner as those with wheat. Sunflower plants were used when the first 2 leaves above the cotyledons had expanded sufficiently to be inoculated.

RESULTS.—*Comparison of the components of RH-1 as eradicative fungicides.*—Table 1 shows that the eradicative properties of RH-1 were due entirely to its content of nickel. The inorganic nickel chloride salt was an effective source of nickel for eradicating stem rust of wheat in the greenhouse. The parent amine was without effect as an eradicative type of fungicide. Results were similar in a test with leaf rust of an unknown race. Furthermore, the eradicative actions of the nickel salt amine complex and nickel chloride were more effective after the leaf epidermis had been broken by developing uredia than before.

Effectiveness of nickel from various sources in protective and eradicative activity.—Since it was possible that other forms of nickel would prove more satisfactory than those already tested, several inorganic sources of nickel were tested as protective and eradicative fungicides. Nickel acetate, chloride, fluoride, nitrate, and sulfate at 70 ppm of nickel were compared with RH-1 at 280 ppm nickel (Table 2).

At 70 ppm nickel, the nickel acetate, sulfate, nitrate, and chloride were all effective sources of nickel for protective action against leaf and stem rusts of wheat. RH-1, either as a crude or a technical preparation, was effective. Nickel fluoride was more effective against stem rust than against leaf rust. Nickel chloride at 70 ppm of nickel, with the addition of either 444 or 1333 ppm of NH_3 as ammonium hydroxide, was without appreciable effect in these particular experiments, in either protective or eradicative action. However, conflicting evidence on this nickel ammonia complex has been reported[5] from rye rust laboratory trials, and unpublished field data indicate fungicidal activity for this complex in 2 instances.

The order of effectiveness of the above sources of nickel as eradicative fungicides for leaf and stem rust of wheat, judged by visual observation, was: RH-1 = nitrate = chloride > sulfate = fluoride = acetate. The greenhouse tests indicated that the concentration of nickel necessary for eradication was higher for stem rust than for leaf rust. The order of effectiveness of the nickel salts for eradication was the same for stem rust as for leaf rust, but 140 ppm nickel was necessary to ensure complete eradication of stem rust whereas 70 ppm was sufficient for leaf rust.

Summary of results for several rusts.—Table 3 presents a compilation of the results of experiments to determine the effective concentrations of nickel, as derived from $NiCl_2 \cdot 6H_2O$, necessary for protective and eradicative action against leaf and stem rust of wheat, crown rust of oats, and sunflower rusts.

Effect of simulated rain.—An eradicative fungicidal spray of 280 ppm nickel was used in the greenhouse against stem rust of wheat 7 days after inoculation of the seedlings. When 25 ml of water was sprayed on each pot of 10 small seedlings 4 hours after application of the fungicide, the eradicative effect was only slightly reduced. However, 100 ml of water 20 hours after fungicide application completely nullified the effect of the fungicide from some of the infections on each leaf. These reductions in activity of the nickel

TABLE 2.—*The protective activity of RH-6[a] and certain inorganic nickel salts against leaf and stem rust on Marquis wheat*

Chemical	Nickel content (ppm)	Average number of infections per leaf	
		Leaf rust	Stem rust
Nickel acetate	70	0.4	0.1
Nickel sulfate	70	0.2	0.0
Nickel nitrate	70	0.1	0.0
Nickel chloride	70	5.9	0.2
Nickel chloride + 1 ml conc. NH_4OH[b]	70	59.4	37.1
Nickel chloride + 3 ml conc. NH_4OH	70	56.1	
Nickel fluoride	70	32.3	0.3
RH-6 crude	280	0.1	0.0
RH-6 technical	280	0.2	0.0
Control	0	76.5	19.3

[a] The nickel salt amine complex bis [*N*-(2-hydroxyethyl) dodecylbenzylamine] nickel (II) chloride.

[b] 70 ppm Ni and 444 ppm NH_3.

TABLE 3.—*Preventive and eradicative fungicidal activity of inorganic nickel ($NiCl_2 \cdot 6H_2O$) against leaf and stem rust of wheat, crown rust of oats, and sunflower rust*

Rust and Host	Effective concentration range of nickel (ppm)		Lowest concentration that damages host (ppm)
	Eradicative action	Protective action	
Puccinia graminis f. sp. *tritici* on Marquis wheat	140–280, ½–1 lb./A.[a]	70–280, ¼–1 lb./A.	280
Puccinia rubigo-vera f. sp. *tritici* on Marquis wheat	70–280, ¼–1 lb./A.	35–280, ⅛–1 lb./A.	280
Puccinia coronata f. sp. *avenae* on Ukraine oats	140–280, ½–1 lb./A.	17.5–280, 1/16–1 lb./A.	70
Puccinia helianthi on S_{37} hybrid sunflower	Effective range causes phytotoxicity	280, 1 lb./A.	280

[a] The estimated rates of application of the $NiCl_2 \cdot 6H_2O$ per acre are based on the field performance of 280 ppm nickel in RH-6 in small field plots in 1956.

occurred whether the nickel was supplied as $NiCl_2 \cdot 6H_2O$, $NiNO_3 \cdot 6H_2O$, or RH-6 (bis[*N*-(2-hydroxyethyl)dodecylbenzylamine] nickel (II) chloride).

When water was applied 1 hour after rust urediospores had been applied to the leaf, 25 ml of water on each pot of 10 small wheat seedlings did not nullify the protective action against leaf rust of 280 ppm nickel, whether supplied as the inorganic salt or as RH-6. However, 100 ml of water nullified the protective action if the nickel was supplied as nickel chloride, though not if supplied as RH-6.

Effectiveness of nickel in relation to the age of the rust infection.—Since, in nature, wheat plants may be subjected to daily showers of urediospores and thus carry infections of various ages, it was important to know at which stages of rust development the eradicative action of nickel is most effective. Nickel at 280 ppm was effective in eradicating stem rust from Little Club wheat seedlings when applied on the 4th, 5th, 6th, 7th, or 8th day after inoculation. It has been found that nickel is without effect in arresting the growth of the fungus if applied after the rust pustule has been sporulating 2 days.

DISCUSSION.—Sempio, in 1936,[6] reported that the leaf rust disease was depressed when wheat plants were supplied through the roots with nickel in *M*/7500 solution. Apparently there have not been any reports on the effectiveness of nickel sprayed on leaves in controlling leaf and stem rust of wheat plants.

Keil, Frohlich, and Glassick[5] reported that nickel compounds will control leaf rust of rye in the greenhouse, by both protective and eradicative action. The present paper reports that nickel compounds will control leaf and stem rust of wheat in the greenhouse by both protective and eradicative action. Furthermore, crown rust of oats and sunflower rust can also be controlled by the protective action of nickel.

The effect of ammonia in preventing the action of the nickel, in both protective and eradicative action, is not understood. However, it is possible that the formation of the complex ion $Ni(NH_3)_6^{++}$ by the interaction of ammonia and nickel hydroxide has the effect of preventing the nickel from acting as a fungicide. However, this hypothesis needs verification, owing to conflicting results obtained by other investigators.[3, 6]

There is obviously less resistance to simulated rain by the soluble nickel salts than by RH-1. The field tests of 1956 revealed that several nickel salt amine complexes were very efficient eradicative fungicides. Since greenhouse tests have shown that these nickel complexes also have protective action, it must be assumed that protective action also played a part in the successful control of leaf rust in the field.

Is the nickel, as supplied by the inorganic nickel salts, likely to be an efficient fungicide under field conditions? The protective action of nickel from soluble inorganic salts is more easily removed by simulated rain than is the protective action of nickel from nickel salt amine complexes. In the field, therefore, a complex may prove much more practical than the inorganic nickel salts.

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[6] Sempio, C. 1936. Influenza di varie sostanze sul parassitamento: ruggine del fagiolo, ruggine e mal bianco del frumenta. Rivista di Patologia Vegetale 21: 201-278.

METHODS OF INFECTING WHEAT WITH THE DWARF BUNT FUNGUS[1]

Jack P. Meiners[2]

SUMMARY

Tests of techniques for producing dwarf bunt in wheat revealed several methods capable of producing high percentages of infection. With most of the methods, germinating spores of *Tilletia contraversa* were placed in contact with seeds, which then were germinated at controlled temperatures. In some cases, germinating spores were applied to seedlings, which then were maintained at definite temperatures while infection took place.

Spraying spores on the soil after shallow seeding in early autumn produced uniformly high percentages of dwarf bunt on susceptible wheat varieties.

INTRODUCTION.—Dwarf bunt (*Tilletia contraversa* Kuehn) is a widespread and serious disease of winter wheat in the Pacific Northwest. Although it has been under more or less intensive investigation for the past 25 years, much is yet to be learned of its nature and the biology of its causal organism. Probably the most important reason for scanty information on dwarf bunt has been the lack of a method of inducing infection on the host. The inability to produce infection by inoculating seed of the host with spores of the fungus, the standard method used in studies of common bunt (*T. caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro), has greatly hindered the study of such aspects as the life history, physiologic specialization, ecology, and host range of the dwarf bunt pathogen, and hindered the breeding of wheat varieties resistant to the disease. The ultimate control of dwarf bunt depends on elucidation of these factors and on a reliable method of producing infection on wheat.

Attempts were begun in 1953 to develop methods of producing infection on wheat with the dwarf bunt fungus. A preliminary report has been published (7).

REVIEW OF LITERATURE.—Several workers have reported that little or no infection results when seed is infested with the dwarf bunt fungus (3, 5, 14). Likewise, seeding wheat in naturally infested soil is unreliable, frequently resulting in little or no infection (4). Generally unsatisfactory results have also been reported when spores of dwarf bunt were mixed with the surface layers of soil before wheat was seeded (5, 13, 14). On the other hand, good infection has been obtained when spores were applied to the soil surface after wheat was seeded (1, 11). In Germany, Röder (11) obtained 32–68% dwarf bunt by this method, and later, in Canada, Baylis (1), using the same technique, secured 63–78% dwarf bunt.

Certain workers have attempted to obtain infection by applying germinated spores to germinating wheat seeds. Gassner and Niemann (2) obtained a maximum of 15.4% infection by spraying winter wheat seedlings that had 2-mm coleoptiles with a suspension of sporidia, and incubating the seedlings 2 weeks at 5°. Inoculating older seedlings with coleoptiles 5–10 mm and 10–30 mm long gave but little dwarf bunt: 1.9 and 0.6%, respectively. In a similar experiment, incubation at 2° resulted in 12.5% infection whereas incubation at higher temperatures, 5° and 12°, was less conducive to infection (3.7 and 1.2%, respectively) (2). Using the same method to inoculate spring wheat, these same investigators obtained a maximum of 16% dwarf bunt when inoculated seedlings were incubated 2 weeks at 5°.

Niemann (9) described in detail a method of producing infection of wheat seedlings to be used in breeding for resistance. He used a suspension of germinating spores containing fused sporidia, and wounded seedlings 2–5 mm long were either sprayed or dipped in the spore suspension, planted in pots containing moist compost, sand, and field soil, and incubated 3–4 weeks at 3–5°. He obtained 40–60% dwarf bunt with this method.

METHODS AND RESULTS.—Germinating dwarf bunt spores, applied to either wheat seed or seedlings, were used in nearly all the studies. The spores were not always from the same source, nor was the wheat variety always the same. Red Bobs spring wheat was used in 1954 and 1955. In 1954 the dwarf bunt spores were from Elgin, Oregon; in 1955 they were from near Nephi, Utah. No studies were carried out in 1956, because the dwarf bunt spores selected for that test failed to germinate. In 1957, Orin wheat was inoculated with spores of a dwarf bunt collection made near Pullman, Washington.

The spores to be used as inoculum were first surface-sterilized by soaking 1 minute in a 5% solution of Clorox (5.25% sodium hypochlorite), and then washed in sterile distilled water. In 1954 and 1955, the spores were germinated in culture dishes on 2½ or 3% water agar in incubating refrigerators held at 5°. Because the spores failed to germinate under these conditions in 1956, the medium was changed to soil extract agar (6) and the spores were incubated in a greenhouse at about 5°. The culture dishes were arranged so that the spores were exposed to daylight and artificial light but shaded from direct sunlight. In most of the culture dishes, 60–75% of the spores germinated after about 6 weeks of incubation. At time of inoculation, the culture dishes contained un-

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germinated spores; germinated spores with promycelia only; single and fused primary sporidia; secondary sporidia; and hyphae. All these components are therefore included by the term "germinating spores."

To eliminate contamination by seed-borne smut spores, all wheat seed, before inoculation, was immersed 15 minutes in a 3:1000 formalin-water solution, washed, and dried.

All inoculated seeds and seedlings were incubated at 5°, 10°, and 15° for varying periods. Then the seedlings not already growing in pots were transplanted to plant bands containing a potting mixture (½ soil, ¼ sand, and ¼ peat moss) and held in the greenhouse until the seedlings were established. They were then placed outside until spring. This outdoor exposure also vernalized the winter wheat. Finally, the plants were transplanted to the field to mature, after which percentages of dwarf bunt were determined on the basis of head counts. All inoculations were made in duplicate.

In the first method to be described, germinating spores were placed on wheat seeds in the following manner: 2 ml of tap water and 100 seeds were placed in a culture dish containing spores germinating on agar, and the seeds were stirred with a glass rod until well covered with a thick spore suspension. The seeds were then placed on a layer of moistened vermiculite 2 cm deep in a preparation dish and covered with an additional 2-cm layer of vermiculite. The preparation dish was covered with a lid and held in an incubating refrigerator until the seedlings had emerged.

In some cases sand, field soil (Palouse silt loam), or a potting mixture was substituted for vermiculite, and seedlings with coleoptiles 1–5 or 5–15 mm long were substituted for seeds. Another variation in the technique consisted of placing seeds in a preparation dish on a layer of vermiculite or other substrate. The layer of agar bearing the germinating spores was removed from a culture dish and inverted over the seeds. These were covered by another layer of vermiculite and incubated until the seedlings had emerged.

In still another method, filter paper was substituted for vermiculite to maintain the proper humidity for infection. In this case the seeds were placed on 2 filter papers moistened with 5 ml of sterile tap water, in the bottom of a culture dish. The agar bearing the germinating spores was then removed from the culture dish in which the spores had germinated, and inverted over the seeds.

Attempts to inoculate spring wheat with germinating dwarf bunt spores by these methods were initiated during the winter of 1953–54. Results were so promising that more extensive experiments were conducted in later years. Table 1 summarizes the results of these experiments.

The percentage of dwarf bunt obtained varied greatly, with differences between replications as well as between inoculation methods. Likewise, the same method sometimes gave different results in different years. Furthermore, the effect of temperature on degree of infection was not consistent. For example, when spores were removed from the agar, placed on the seeds, and incubated in vermiculite, the highest average percentages of bunt occurred at 15° in 1954, at 5° in 1955, and at 10° in 1957 (Table 1).

Apparently, the substratum used greatly influenced the degree of infection. Vermiculite or filter paper produced higher smut percentages than did field soil or greenhouse potting soil. Sand gave inconsistent results (Table 1).

In many cases, factors other than temperature appeared to influence infection. For example, in the methods in which the agar layer bearing spores was inverted over seeds and incubated in soil or potting mixture, much greater smut infection was produced when the seeds were incubated in the greenhouse at 5° than when incubated at 5° in the refrigerator.

A variation on the technique using filter paper gave considerable success: Seeds were placed on moistened filter paper in the top of a culture dish, the bottom of which contained a layer of agar bearing the germinating spores. The plate was then incubated in an inverted position until the seedlings were 3–5 cm long. This method produced 40–60% dwarf bunt at the 5° incubation temperature. This procedure was simpler and quicker than those described above.

Another technique utilized the agar upon which the spores were germinated as a substratum for seed germination. In this procedure, wheat seeds were pressed into agar on the surface of which dwarf bunt spores were germinating, with the embryo in juxtaposition to the spores. As the wheat germinated, the position of the culture dishes was changed so that the coleoptiles would grow along the surface of the medium. Following inoculation, the dishes were maintained at 5, 10, and 15°C until the coleoptiles of the seedlings were 3–5 cm long. Maximum smut percentages at 5, 10, and 15° were 44, 25, and 62%, respectively, on Red Bobs wheat in 1955, and 40, 28, and 50%, respectively, on Orin wheat in 1957.

Rowell and DeVay (12) developed a method for inoculating corn seedlings with isolates of *Ustilago maydis* (DC.) Cda., in which the tip of the coleoptile was clipped from seedlings, which were then placed in the inoculum and subjected to partial vacuum. This technique was modified and used to inoculate wheat seedlings with germinating spores of dwarf bunt. Fifty seedlings were germinated until the coleoptiles were 1–2 mm long. These were then put in a test tube, covered with a water suspension of germinating spores, and subjected to 5 minutes of partial vacuum obtained with a water aspirator. The seedlings then were placed in vermiculite in a preparation dish and incubated until they were 3–5 cm long. In a parallel series, 1 mm of the tip was clipped from seedlings 2–4 mm long before subjecting them to 3 minutes of partial vacuum. The results (Table 2) show that this

method could produce high percentages of dwarf bunt at all 3 temperatures of incubation. However, results varied somewhat among replications.

In an attempt to duplicate the inoculation method of Gassner and Niemann (2), wheat seedlings with coleoptiles 2–4 mm long were placed on moist sand in 4-in. pots and sprayed with a water suspension of germinating spores. The seedlings were then covered with 1 in. of moist sand, and the pots were put in plastic bags to prevent loss of moisture, and incubated at 2°, 5°, 10°, and 15° until the seedlings emerged. Smut percentages at 2° and 5° were 34 and 13, respectively. These results compared favorably with those of Gassner and Niemann, who obtained 12.5 and 16% bunt at the respective temperatures. At 10° and 15° the maximum smut percentages were 44 and 21, respectively.

Attempts were made to inoculate wheat seedlings after emergence. In this procedure, wheat seedlings in 4-in. pots were sprayed with a water suspension of germinating spores at 3 stages of seedling development: 1-leaf, 2–3-leaf, and 4-leaf to tillering. After inoculation, the pots were placed outside until spring, when the seedlings were transplanted to the field. The results of the inoculations are presented in Table 3. Percentages of dwarf bunt were somewhat lower than with methods described earlier in this paper. Neither stage of development nor temperature at which the seedlings were held after inoculation appeared to influence the degree of infection.

A number of methods involved germinating spores in or on the soil, and seeding wheat in this soil or on layers of germinating spores on the soil. Little or no infection resulted, despite the fact that this technique probably more nearly approaches natural conditions than do some of the more effective methods tried.

Another technique for producing infection on winter wheat, under test at Pullman, is similar to the one

TABLE 1.—*Dwarf bunt obtained on 2 wheat varieties by applying germinating spores to seeds and seedlings and incubating on various substrata at various temperatures*

			Per cent dwarf bunt[b]					
			Red Bobs wheat				Orin wheat	
			(1954) Replication		(1955) Replication		(1957) Replication	
Substrate	Stage inoculated	Incubation °C[a]	I	II	I	II	I	II
METHOD: *germinating spores placed on seed*								
Vermiculite	Seeds	5	0	3	91	61	15	10
		10	6	31	26	40	81	65
		15	43	12	24	12	48	37
Sand	Seeds	5	9	7	53	70	0	0
		10	0	12	45	39	0	0
		15	20	12	16	20	0	0
Soil	Seeds	5	12	6	8	10	0	2
		10			11	1	0	2
		15			5	10	0	3
Vermiculite	Seedlings (1–5 mm)	5					95	0
		10					45	11
		15					50	12
Vermiculite	Seedlings (5–15 mm)	5					18	80
		10					9	15
		15					19	7
METHOD: *spore-bearing agar inverted over seeds*								
Vermiculite	Seeds	5					100[c]	40
		10					46	40
		15					0	38
Soil	Seeds	5					2	6
		10					0	0
		15					0	5
		5[d]	21	14			34	50
Potting mixture	Seeds	5			46	7	15	0
		10			15	4	0	1
		15			6	7	0	4
		5[d]					33	46
Filter papers	Seeds	5			60	68	25	20
		10			5	27	14[c]	34
		15			15	0	100[c]	23

[a] The temperature at which seeds or seedlings were maintained after germinating spores were placed in contact with them. Incubation carried out in incubating refrigerators unless otherwise noted.
[b] Not inoculated if no percentage shown.
[c] Poor stand.
[d] Incubated in greenhouse at about 5°C.

described by Röder (11), in 1953, and by Baylis (1), in 1955. The technique, as developed over a 3-year period beginning in 1954, was as follows: Winter wheat was seeded in the field early in the fall (late September or early October) at a depth of 0.5–1.0 in. Then a heavy suspension of dwarf bunt spores was applied to the soil surface above the seeds by means of an ordinary sprinkling can or knapsack sprayer.

Applying dwarf bunt spores to the soil surface after shallow seeding gives substantially higher infection than does naturally infested soil or spores artificially incorporated into the soil. Test plots of Orin winter wheat seeded in the fall of 1954 showed 3 and 6% dwarf bunt infection without surface application, and 12–15% with surface application. Identical inoculations made the following year produced dwarf bunt infection percentages of 14 and 42, respectively, for non-surface and surface applications. In 1956–57, this method was used extensively at Pullman in pathogenic race, life history, and ecological studies of the dwarf bunt fungus, and for varietal testing of wheat for resistance. Up to 80% dwarf bunt was obtained on susceptible wheat varieties by this method.

Discussion.—Some of the methods described here for producing dwarf bunt in wheat appear effective and reliable, and not excessively laborious. When relatively large quantities of inoculum are available, spraying spores on the soil after seeding provides ample infection for testing spore collections for pathogenic race affiliation, and wheat varieties for resistance to dwarf bunt races. This method has also been effectively used to provide uniformly high infection of dwarf bunt for experiments on control with fungicides (10). Studies in progress on the life history of dwarf bunt have used this technique for obtaining infection. The same method was used to produce dwarf bunt infection on grasses in a study of the host range of the organism (8).

If the spore material available for experiments consists of only a few smutty heads, then germinating spores must be used. The method in which spores are removed from agar and placed on seeds that are then allowed to germinate in vermiculite at 5° has been effective in pathogenicity studies of such collections. In 1957, 16 of 20 collections tested for race affiliation by this method were positively identified as to pathogenic race. In this series, dwarf smut percentages of 75 or over were common on susceptible wheat varieties. To compare this method with the technique of spraying spores on the soil surface after seeding, collections of dwarf bunt spores were tested for pathogenic race using both methods when abundant spore material was available. Results compared favorably, but the former technique was more laborious, since it is necessary to transplant the wheat seedlings twice in the process. The great variation in infection between replications (apparent in Table 1) did not occur in these large-scale tests.

Other techniques of using germinating spores that have given high percentages of dwarf bunt should be useful in large-scale tests with dwarf bunt collections. Among these are methods in which culture dishes are used, either by employing filter papers to maintain moisture while the seeds are germinating or by pressing seeds into the agar on which spores are germinating. Because culture dishes take up little space, many of them can be placed in an incubator after inoculation. The partial-vacuum method of inoculating seedlings might be useful, although it takes longer than most of the methods described here.

The cause of variations between replications in degree of infection is difficult to determine. Since each inoculation represents a different culture dish of germinating spores, it is possible that irregular spore germination may be a cause of the variation observed. Differences in germination were disclosed by microscopic examination of the culture plates, and this result emphasizes the need for uniformity of inoculum if results are to be consistent.

Just why vermiculite has given better and more consistent results than have other substrates is a

Table 2.—*Dwarf bunt obtained on 2 wheat varieties by subjecting seedlings to partial vacuum in a suspension of germinating spores*

Coleoptile length	Incubation °C[a]	Per cent dwarf bunt: Red	Bobs, 1955 Replication I	Bobs, 1955 Replication II	Orin, 1957 Replication I	Orin, 1957 Replication II
1–2 mm	5		13	26	31	27
	10		50	26	3	35
	15		54	13	0[b]	19
2–4 mm[c]	5		59	2	18	61
	10		27	49	58	45
	15		16	7	24	61

[a] The temperature at which seedlings were maintained after germinating spores were placed in contact with them. Incubation carried out in refrigerators.
[b] Poor stand.
[c] Tip of coleoptile clipped off.

Table 3.—*Dwarf bunt obtained on 2 wheat varieties by spraying seedlings with germinating spores*

Growth stage	Incubation °C[a]	Per cent dwarf bunt[b]: Red	Bobs, 1955 Replication I	Bobs, 1955 Replication II	Orin, 1957 Replication I	Orin, 1957 Replication II
1-leaf	5		0	0	2	28
	10		0	1	16	1
	15		0	0	0	15
2–3-leaf	5		0	7	0	1
	10		2	0	2	10
	15		2	0	6	12
4-leaf to Tillering	5				5	0
	10				0	11
	15				1	2

[a] Temperature at which seedlings were maintained after germinating spores were sprayed on them. Incubated in incubating refrigerators.
[b] Not inoculated if no percentage shown.

matter of conjecture. Possibly, the moisture content of this medium is more uniform than that of the other substrates used; or the physical structure of vermiculite provides better conditions of aeration and, therefore, more favorable environment for growth and for infection of the host by the pathogen. Vermiculite also is more uniform in chemical composition and pH than are sand, soil, and potting mixtures.

The spraying of seedlings in the 1-leaf to tillering stages with germinating spores resulted in significant amounts of infection in all stages of seedling development. Although the amounts of dwarf bunt obtained with these methods do not compare favorably with those of other methods, they indicate that wheat plants are susceptible to infection by the dwarf bunt fungus for a considerable period after emergence. Thus, it is possible that the life cycle of dwarf bunt includes some post-emergence infection, and, if so, this could be a complicating factor in its control.

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STRAIN AND SEED TREATMENT AS FACTORS IN GERMINATION AND SEEDLING GROWTH OF SMOOTH BROMEGRASS[1]

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SUMMARY

Seed treatment with fungicides of varieties and experimental synthetics of smooth bromegrass were conducted on Plainfield fine sandy loam. Differences early in the 1953 growing season had disappeared by the time of the autumn digging; those in the early growing season of 1954 persisted to a greater degree, although differences among treatments became less as the season progressed. Organisms isolated from necrotic host tissue were primarily those known to incite seed rotting, root rot, or seedling blight of brome. *Helminthosporium sativum*, *Fusarium roseum*, and *Pythium graminicola* were the most abundant both seasons. *F. oxysporum* and *Rhizoctonia* spp. were present later in the season. *H. bromi* incited leaf spot and seedling damage in 1953, but not in 1954.

The slow establishment and development of satisfactory stands of smooth bromegrass (*Bromus inermis* Leyss.) are problems of considerable concern on many Wisconsin farms, particularly on the lighter, sandy soil of the central and western portions of the state, though the problems are also frequently encountered in areas characterized by heavier soil types.

Exploratory greenhouse studies employing captan (Orthocide) as a seed fungicide yielded encouraging results in seedling growth when duplicate plantings of 100 seeds each of 32 seed lots of 16 named varieties and commercial lots, and of 16 experimental synthetics were made in Plainfield sand from the Hancock Experiment Station. Seeds treated at ¼ ounce of captan per bushel averaged 74.9% emergence; untreated seeds averaged 49.7%. Accordingly, experiments were undertaken to determine whether such treatments were effective in improving stand establishment in the field.

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LITERATURE REVIEW.—Investigations directed toward understanding the causes of poor stand development of smooth bromegrass have been relatively infrequent. Newell and Keim (6), at Lincoln, Nebraska, demonstrated differential performance of strains of northern and southern origin. Hawk and Welch (4) reported that late seedings in western Iowa and in South Dakota caused losses of seedling stands. Investigations by Sprague (7) and Gordon and Sprague (3) indicated as pathogens on seedlings and roots: *Helminthosporium sativum* Pam., King & Bakke (also known as *H. sorokinianum* Sacc.), *Pyrenochaeta terrestris* (Hanson) Gorenz, J. C. Walker & Larson, *Colletotrichum graminicola* (Ces.) G. W. Wils., *Gleosporium bolleyi* Sprague, and species of *Pythium*, *Fusarium*, and *Rhizoctonia*. Sprague (8) considered that *Helminthosporium bromi* Died. occurred incidentally on roots of *Bromus* species. Andrews (1) found that several of the pathogens listed by Sprague were associated with seedling blight of grasses, including smooth bromegrass. Thomason (9) determined that soil type and temperature influenced stand development by Arasan-treated and untreated seed in naturally infested soil. Total seedling emergence was not influenced by temperatures varying from 16° to 28°C. He isolated the fungi from blighted seedlings and determined their pathogenicity. The pathogens most commonly isolated included *H. sativum* and species of *Pythium* and *Rhizoctonia*. The literature dealing with root disease fungi and the effect of seed treatments on stand and yield has been summarized by Garrett (2), Kreitlow, Garber, and Robinson (5), and Tyler, Murphy, and MacDonald (10).

MATERIAL AND METHODS.—Experimental plantings were made on Plainfield fine sandy loam at the Experiment Station at Hancock, Wisconsin, during the springs of 1953 and 1954. Leaf spot incited by *H. bromi* was moderately abundant on the foliage of smooth bromegrass plants growing in a grass roadway immediately adjacent to the experimental plot in 1953, but not in 1954.

The soil of the trial area had been used before 1953 for general experiments with corn, small grain, and grasses. At the time of the 1954 planting, the soil tested: pH 6.0; 150 lb. N, 60 lb. P_2O_5, and 240 lb. K available per acre. The 1953 planting had been made in an adjoining site in the same field. Although determinations were not made the first year, it may be assumed that the soil factors were essentially similar to those of 1954 since these adjacent areas had received similar cultural and fertilizer treatments during previous years.

Thirty-two seed lots of 16 named varieties and commercial sources, and 16 experimental synthetics were included in the 1953 planting. The named strains and commercial lots included:

Canadian Common (2 lots)	Superior (2 lots)
Manchar	Lincoln
Southland	Fischer
Martin	Homesteader
Sandberg	B. in 12 (2 lots)
Lancaster	Elsberry (2 lots)
Oklahoma No. 1	Mandan 404
Lyon	Achenbach

The experimental synthetics included 15 developed at the Wisconsin Agricultural Experiment Station and 1 (BR-3) developed at the Iowa Agricultural Experiment Station.

Six varieties (Homesteader, Lincoln, Fischer, Mandan 404, Martin, and Canadian Common) were included in the 1954 planting.

In both trials 100-seed lots were planted in rows 3 ft. long and 12 in. apart, with furrows 1 in. deep. Following placement by hand, the seeds were covered carefully and the soil firmly tamped.

Five replications were employed both years, in a 4×8 lattice design in 1953, and in a split plot design in 1954. Both years, seed lots were arranged in whole plots and fungicides in sub-plots. Seed treatments in 1953 included dusting with captan (Orthocide, 75% *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide); Ceresan M (7.7% *N*-(ethylmercuri-*p*-toluenesolfonanilide); and Arasan (75% bis(dimethylthiocarbamoyl) disulfide) individually, at ¼ oz. per bu. of seed, and an untreated check. The 1954 treatments included dusting with captan at ¼ oz. per bu., dusting with Arasan at ¼ oz. per bu., placing an excess of Arasan in the furrow with the seed at planting, and a check of untreated seed.

The 1953 planting was made on May 9. Stand counts were determined on all 5 replications on May 28 and June 25. On September 18, seedlings were dug and examined for the number of diseased and healthy plants in the first 2 replications.

The 1954 planting was made on April 3. Stand counts were made on May 22 and on June 9. Three replications were dug on September 23, and the numbers of healthy and diseased seedlings recorded. Both years, representative plants were selected at digging to determine the pathogenic organisms involved.

Data on disease incidence and pathogens were obtained by customary methods. Differences between seedling stands in treated and untreated rows indicated losses, either from phytotoxic action of the fungicides or from seed rotting and seedling blight before or soon after emergence. Diseased seedlings, determined after the seedlings were dug, included dead plants and those showing necrosis of roots and crowns. Brown discoloration of the roots without cortical necrosis was not included. Sections about ½ in. long of roots and crowns were surface-sterilized ½ minute in equal parts of commercial sodium hypochlorite (3%) and ethyl alcohol (50%) and plated on water agar and potato-dextrose agar. The organisms isolated, listed below, have been frequently tested for pathogenicity on smooth bromegrass (9).

The data were analyzed by analysis of variance. Summary tables include the results of the 2 trials.

RESULTS.—*1953 planting.*—Soon after emergence, seedling plants began to exhibit various degrees of disease infection, particularly that caused by *H. bromi*. Other organisms, if present, were of minor importance. Plants within the experimental area appeared very uniform during the early growth, but quite prominent differences developed at the 4–5 leaf stage. These were partly associated with variety, but appeared primarily due to local soil variations, for differences among replications were striking.

Data compiled on May 28 indicated that the number of seedlings following treatment with Ceresan M was generally lower (exceeded LSD at 5%) than that following treatments with other fungicides, in which the stands were essentially similar. Strains of northern origin appeared to have greater susceptibility to seedling diseases than did strains from southerly states.

The data indicate that highly significant differences occurred among strains, replications, and treatments at each of the dates of examination. Increase of time from planting date lessened the interaction between strains × treatments until September 18 (termination of the experiment), when the interaction had become non-significant.

During late summer many seedlings appeared weak, and had made little growth. Differences between replicate plantings had become smaller, with apparent infection areas prominent across replications and irrespective of variety. Especially noticeable was the occasional occurrence of vigorous, healthy-appearing seedlings, somewhat at random, in the area. Such plants may have been more resistant to seedling blights, and possibly to other diseases, or they may have been escapes from disease infection.

Organisms isolated from necrotic host tissue indicated that greatest damage was done by *H. sativum* and *Pythium graminicola* Subr., which were most prevalent. *Rhizoctonia* spp. were present in some abundance, but appeared of lesser importance, based on the type of lesions from which they were isolated as compared to the organisms previously mentioned, and on pathogenicity tests. Isolates of the *Fusarium roseum* (Lk.) Snyder & Hansen complex were prevalent at the earlier date; isolates of *F. oxysporum* Schlecht were prominent later. The latter has been shown to be pathogenic in roots at higher temperatures (9), but may perhaps best be regarded as inciting secondary infections.

1954 planting.—Poorer stands developed from untreated seed lots, except Fischer, than from seed lots treated with fungicides. Fischer seed treated with captan produced fewer seedlings (not significant) than Fischer treated with other fungicides. Otherwise, there appeared to be relatively little difference among treatments. This suggests that differences found among strains were largely contributed by the check treatment. Comparison of the check and the average effect of seed treatment of all strains was not consistent upon the first reading date, for which a significant F-value (4.46**) was obtained. Comparison of the means for varieties suggests that strains of northern origin (Martin, Mandan 404, and Canadian Common) tended to derive more benefit from treatment than did those of more southerly or drier locations (Lincoln, Fischer, and Homesteader). However,

TABLE 1.—*Analysis of variance of seedling stand percentages for smooth bromegrass subjected to different seed treatments (field-seeded at Hancock, Wisconsin, 1953 and 1954)*

1953	May 28		June 25		Sept. 18	
	df	MS	df	MS	df	MS
Source of variation						
Replications	4	850.99**	4	3030.12**	1	7832.25**
Strains	31	1383.04**	31	882.47**	31	1004.36**
Error *a*	124	138.64	124	258.98	31	219.85
Seed treatments	3	8660.72**	3	1634.57**	3	617.82**
Strains × treatments	93	136.27**	93	95.51*	93	46.09
Error *b*	384	84.57	383*	69.97	96	50.23
Coef. var.	15.2%		25.3%		36.0%	
1954	**May 22**		**June 9**		**Sept. 23**	
	df	MS	df	MS	df	MS
Source of variation						
Replications	4	61.92	4	131.72**	2	182.39
Strains	5	4047.85**	5	3571.35**	5	2157.91**
Error *a*	20	36.46	20	36.99	10	136.87
Seed treatments	3	536.12**	3	473.66**	3	135.53*
Strains × treatments	15	62.48	15	50.36	15	47.45
Error *b*	72	38.57	72	35.61	36	42.22
Coef. var.	9.2%		9.0%		13.2%	

* One missing value estimated.
* significant at 5% level.
** significant at 1% level.

TABLE 2.—*Combined analysis of variance for seedling counts of smooth bromegrass subjected to different seed treatments (Hancock, Wis, 1953 and 1954)*

	May 28 and June 25, 1953		May 22 and June 9, 1954	
	df	MS	df	MS
Source of variation				
Replications	4	3348.50**	2	101.12
Strains	31	1865.69**	5	6004.39**
Error *a*	124	251.15	10	149.57
Dates	1	232174.31	2	7692.35**
Strains × dates	31	399.81**	10	267.12**
Replications × dates	—	—	4	111.78*
Error *a'*	128	158.54	20	34.86
Treatments	3	8890.24**	3	887.79
Treatments × strains	93	152.03*	15	100.03
Error *b*	384	106.92	36	54.28
Dates × treatments	3	1405.05**	6	36.66
Dates × treatments × strains	93	79.75**	30	20.23
Error *b'*	383[a]	47.56	72	27.10
Total	1278[a]	478989.98	215	59815.77

[a] One missing value estimated.
* significant at 5% level.
** significant at 1% level.

these differences were not found at later dates.

Differences among seed treatments are indicated by the May 22 analysis. Essentially the same pattern is apparent in the data of June 9. The lack of conformity in data for the 2 years is considered below.

The fungi associated with seedlings were similar during the 2 seasons, although the relative prevalence of the groups differed considerably. *Pythium* spp. were prevalent on seedling crowns and roots both years. Seedling lesioning and blighting were much less in 1954 than in 1953, although the same *Pythium* species were present both years. *H. sativum*, prevalent in blighted seedlings later in the 1953 growing season, was isolated less frequently in 1954, and resulted in fewer diseased seedlings being blighted in the latter year. *H. bromi*, isolated in 1953, was not found in 1954. Isolations from brown root and crown lesions during the late summer included *F. roseum*, *Helminthosporium* spp., and *Rhizoctonia* spp., in descending order of prevalence.

DISCUSSION.—Although intensity of disease development differed some during the 2 years, the results followed essentially similar patterns. In 1953, the differences between treatments during the earlier portion of the growing season had disappeared by the time of the autumn digging; in 1954 the differences observed during the early growing season persisted to a greater degree. Factors contributing to this differential behavior have not been studied in detail, but several causes may have been contributory to the results. For example, in 1953, adequate moisture and cool temperatures occurred at seeding time and during early seedling development, and a prolonged drought and high temperatures followed. In 1954, the early growing season was very similar to that of 1953, but moderate temperatures followed, with rains sufficiently frequent to maintain growth. Even though the growing season of 1954 was more favorable, differences among treatments became less as the season progressed. This could be interpreted to indicate that growth was sufficient so that a high proportion of disease-infected plants were not eliminated prior to the September digging. However, other factors contributing to plant elimination are possible.

The large "mean squares" obtained for replications, and their generally increasing magnitude as the seasons advanced, especially prominent for the 1953 data, bear out general observations made over a number of years (Tables 1 and 2). Seedling stands of this species usually exhibit patchy and otherwise irregular patterns, suggesting areas of soil infestation. Occasional seedlings, either resistant or those escaping infection, occur in areas otherwise barren. Under more generally favorable conditions of moisture and temperature, such as those experienced during 1954, a growth sufficient to maintain a fair proportion of developing plants ahead of the impact of infection may result in greater differences among strains and seed treatments. These factors could account for the greater differences among seed treatments in 1954 than in 1953.

There is a tendency for southern-adapted strains to exhibit slightly greater, though somewhat variable, tolerance to diseases. This could be interpreted as an indication of the role that soil-borne fungi may have played in ecotype differentiation. The occurrence of occasional vigorous and otherwise healthy-appearing plants suggests the possibility in breeding programs of artificial selection for resistance to seedling disease organisms. Incorporation of such resistance could considerably improve seedling vigor and density and rapidity of stand development.

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RELATION OF FUNGICIDAL AND NEMATOCIDAL BULB TREATMENTS TO PHYTOTOXICITY AND CONTROL OF BASAL ROT IN NARCISSUS[1]

Robert Aycock

SUMMARY

Despite discoloration and apparent injury to narcissus bulbs, Dowicide B was equal or superior to Mersolite W in controlling Fusarium basal rot. Steeping freshly harvested bulbs in Dowicide B solutions caused very conspicuous discoloration that would reduce the value of bulbs where appearance is important. Delaying treatments 7 days after harvest made them relatively ineffective in disease control; delaying treatments 2 weeks after harvest reduced discoloration to negligible. The First variety is much more susceptible to basal rot and chemical injury than are the Flower Carpet and King Alfred varieties.

Introduction.—Basal rot caused by *Fusarium oxysporum* Schlecht f. *narcissi* Snyder & Hansen is the most serious disease of narcissus in southeastern North Carolina. Various fungicidal dips to control this disease have been used before planting and after harvest. Certain treatments showing promise against basal rot were recently found to be lethal to larvae and eggs of the soybean cyst nematode, *Heterodera glycines* Ichinohe (9), discovered in North Carolina in 1954 (10) and since found in about 2000 acres of the commercial bulb-growing area.

Currently recommended treatments to control basal rot have caused general dissatisfaction, and eradicant treatments for the soybean cyst nematode may cause injury. Growers are consequently confused as to proper control measures. This paper reports the results of a series of experiments initiated in 1955 to determine how various bulb treatments affect basal rot control, yield of flowers and bulbs, and phytotoxicity in leading commercial varieties.

Review of literature.—According to McClellan (5), losses from basal rot in North Carolina are often 50% or higher. The greater severity of the disease in coastal regions of the eastern United States is related to warmer temperatures than in other bulb-growing areas. Controlled laboratory and greenhouse experiments (5) have shown that basal rot in growing plants develops most rapidly when soil temperatures are maintained at 70°F, and maximum disease in storage occurs at 85°F.

Bulbs are infected through roots and basal plate in late spring before harvest, and through dead roots if bulbs are left over summer in the soil (4). Further infections are developed from inoculum disseminated during harvest.

In the Pacific Northwest (2, 3) Ceresan (2% ethylmercury chloride), Ceresan M (7.7% *N*-(ethylmercuri)-*p*-toluenesulfonanilide), and phenylmercury acetate were found effective in controlling basal rot. In recent years phenylmercury acetate has been generally used (3, 6) in both eastern and western commercial bulb-growing areas. In North Carolina phenylmercury acetate, sold as Mersolite W, has been recommended and used for both pre-storage and pre-planting treatments.

Recent discovery of the soybean cyst nematode in the narcissus-producing area of North Carolina (10), resulting in a quarantine, created a number of new problems for commercial growers. Skotland (7) demonstrated that, although this nematode was not parasitic on bulb crops, cysts might be spread in infested soil adhering to bulbs after harvest. To develop means of preventing such spread, Winstead and Skot-

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land (9) tested various chemicals that had proved effective or promising in the control of basal rot. Mersolite W and New Improved Ceresan (5% ethylmercury phosphate) dips were not effective at rates recommended for basal rot control. Various concentrations of Dowicide B (85% sodium 2,4,5-trichlorophenoxide), however, were lethal to eggs and larvae when cysts were steeped in them. A concentration of 6 lb. per 100 gal. of water for 30 minutes caused superficial injury and discoloration. No other treatments effective against the nematode caused injury. Observations were based on mature bulbs of the Helios variety treated 2 weeks after July harvest.

North Carolina growers objected to a recommendation (8) that narcissus bulbs be treated with Dowicide B (3 lb. per 100 gal. of water for 15 minutes) to eradicate the soybean cyst nematode. The possibility of discoloration of freshly harvested bulbs, they felt, might reduce the market value of bulbs, especially in the retail market.

MATERIALS AND METHODS.—Single-nose bulbs of the 3 varieties King Alfred, The First, and Flower Carpet were used in these tests. The bulbs available for a given test were distributed as uniformly as possible into the required number of samples. Treatments were replicated 4 times, both in storage and in the field. Split-plot designs were used so that data could be compared from different pre-storage and pre-planting combinations. Cultural practices were comparable to those employed by commercial growers. Bulbs were harvested in May, treated in open-mesh bags within 48 hours, and stored during the summer in 4-qt. tills in wire-bottom trays in an open shed. They were removed from storage in September, and cleaned and treated again a few days before planting. Data obtained include weight and number of bulbs harvested, number with basal rot, yield of flowers, and chemical injury to flowers and bulbs.

EXPERIMENTAL RESULTS.—*King Alfred variety.*—Eleven pre-planting steeps were applied to bulbs. After harvest each of these lots was subdivided into 3 equal groups. One group was treated with Mersolite W (1 lb. per 400 gal. water for 10 minutes), and the second with Dowicide B (3 lb. per 100 gal. water for 15 minutes); the third served as an untreated control. Data on these lots were obtained through 3 growing and storage seasons.

The bulbs that received Dowicide B and Mersolite W pre-storage treatments developed significantly less basal rot than untreated bulbs, but did not differ significantly from each other (Table 1). The mean of all pre-planting treatments showed that there was little difference among most of the treatments in incidence of basal rot. Some carryover through storage was observed from pre-planting treatments with Dowicide B (6 lb. per 100 gal. water for 2 hours) and captan (12 lb. per 100 gal. water for 30 minutes) when no pre-storage dips were given. The pre-planting treatment that resulted in the greatest percentage of basal rot after storage was with Mersolite W.

Dowicide B pre-storage treatments yielded significantly more flowers than did Mersolite W (1 lb. per 400 gal. water for 10 minutes) or untreated bulbs. In yield of bulbs the 2 treatments were superior to control lots but did not differ significantly from each other.

The mean yields of flowers and bulbs of most pre-planting treatments were significantly greater than

TABLE 1.—*Incidence of basal rot (% transformed[a] to angle) in King Alfred narcissus as affected by various combinations of pre-planting and pre-storage treatments with fungicidal dips*

Pre-planting treatments[b]	Pre-storage treatments[b]			Mean
	Mersolite W (1/400, 10 min.)	Dowicide B (3/100, 15 min.)	None	
Dowicide B, 6/100, 2 hr.	9.5	3.2	3.1	5.2
Captan 12/100, 30 min.	0	7.7	8.9	5.5
Ceresan M 1/100, 5 min.	12.7	3.2	16.4	10.8
New Improved Ceresan 2/100, 5 min.	9.5	11.5	12.9	11.3
Emmi 20 ml/gal., 15 min.	7.3	10.7	15.9	11.3
Dowicide B 3/100, 15 min.	5.9	10.2	18.0	11.4
Dowicide B 3/100, 30 min.	13.3	9.0	18.7	13.7
Emmi 10 ml/gal., 5 min.	16.4	11.4	20.8	16.2
Merculine 50 ppm, 15 min.	19.3	12.0	20.5	17.3
Merculine 400 ppm, 15 min.	18.6	11.0	24.1	17.9
Mersolite W 1/400, 10 min.	19.9	12.8	22.3	18.8
None	27.0	21.2	22.7	23.6
LSD (.05)	12.8	12.8	12.8	6.6
LSD (.01)	16.6	16.6	16.6	8.9
Mean for pre-storage	13.4	11.1	18.0	
LSD for pre-storage treatments, .05 = 3.0, .01 = 4.0				

[a] Before statistical analysis, percentages were transformed to angles: angle = arc sin $\sqrt{\text{percentage}}$.

[b] Unless otherwise indicated, concentrations are in lb. per 100 gal. water. Active ingredients of chemicals are: Dowicide B, 85% sodium 2,4,5-trichlorophenoxide; captan, 50% *N*-trichloromethylthio)-4-clyclohexene-1,2-dicarboximide; Ceresan M, 7.7% *N*-(ethylmercuri)-*p*-toluenesulfonanilide; New Improved Ceresan, 5% ethylmercury phosphate; Emmi, 1,4,5,6,7,7-hexachloro-*N*-(ethylmercuri)bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide; Merculine, 10% phenylmercury salicylate; Mersolite W, 96.5% phenylmercury acetate.

those of the control. Exceptions were lots dipped in either of 2 concentrations of Merculine (10% emulsifiable concentrate of phenylmercury salicylate). Yields of bulbs and flowers were greatest from bulbs treated before planting with Dowicide B (3 lb. per 100 gal. water for 15 or 30 minutes).

Data on visible injury from pre-storage treatments were taken at cleaning time. Dowicide B always caused some visible staining and discoloration; Mersolite W caused occasional slight discoloration. The percentage of injured or discolored bulbs from the 2 treatments was 37.3 and 2.6, respectively.

The First and Flower Carpet varieties—When Dowicide B was recommended for control of the soybean cyst nematode (8, 9) the present work was expanded to determine varietal reaction to the treatment, particularly with respect to basal rot and phytotoxicity. Various concentrations for different periods were compared with the recommended Mersolite W pre-storage treatment. An additional pre-storage treatment was included, using the organic mercurial Emmi (10% 1,4,5,6,7,7-hexachloro-*N*-(ethylmercuri) bicyclo-[2.2.1]hept-5-ene-2,3-dicarboximide). After the first storage season each treatment lot was subdivided so that half was treated with Mersolite W before planting and half was treated with Dowicide B (3 lb. per 100 gal. water). The First was found a great deal more susceptible than Flower Carpet to basal rot and phytotoxicity. Table 2 therefore shows data only on The First variety.

At the end of the first storage season untreated lots of The First showed a 40% incidence of basal rot. Differences between treatments were highly significant. The best dip treatment was Dowicide B at 6 lb. per 100 gal. water for 5 minutes. Only 8.5% of these showed rot. Bulbs treated with Mersolite W were not significantly lower than untreated bulbs in basal rot incidence. The same trend in basal rot incidence was noted in Flower Carpet, though generally lower than in the former.

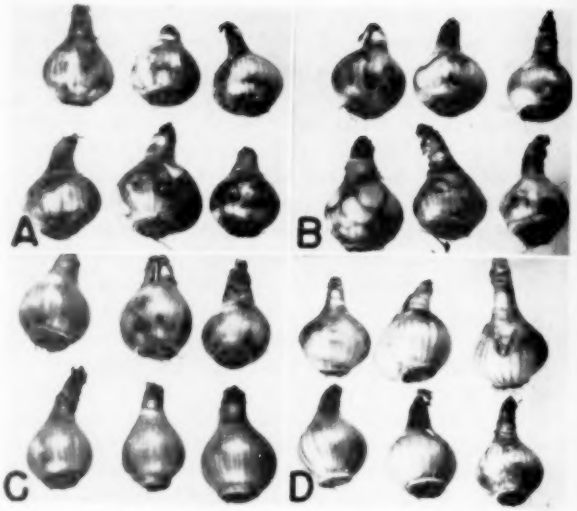

FIG. 1. Chemical injury to narcissus bulbs of The First variety. Photographs made in September after cleaning and removal of outer scales. A) Steeped 48 hours after harvest, in 3 lb. Dowicide B per 100 gal. water for 15 minutes. B) Steeped 48 hours after harvest, in 6 lb. Dowicide B per 100 gal. water for 15 minutes. C) Control. D) Steeped 2 weeks after harvest, in 3 lb. Dowicide B per 100 gal. water for 15 minutes.

TABLE 2.—*Mean yield*[a] *per plot of healthy bulbs of narcissus variety, The First, as affected by various combinations of pre-storage and pre-planting treatments with fungicidal dips to control basal rot*

Pre-storage treatments[b]	Preplanting treatments[b]		Mean
	Mersolite W (1/500, 10 min.)	Dowicide B (3/100, 15 min.)	
	(g)	(g)	(g)
Dowicide B, 6/100, 5 min.	705	626	666
Dowicide B, 6/100, 15 min.	606	618	612
Dowicide B, 3/100, 10 min.	607	614	610
Dowicide B, 3/100, 15 min.	621	537	579
Dowicide B, 3/100, 5 min.	590	531	561
Mersolite W, 1/500, 10 min.	455	524	490
Control	441	399	420
Emmi, 20 ml/100, 15 min.	380	401	391
LSD (.05)			102
LSD (.01)			138

Differences between means of pre-planting treatments not significant (.05)

[a] Yield data recorded September, 1957—after storage (summer of 1956), growing season (Sept., 1956, to May, 1957), and storage (summer of 1957). Initial number of bulbs per pre-storage treatment was 132.

[b] Pre-storage treatment applied 1 day after harvest. Unless otherwise indicated, concentrations are lb. per 100 gal. water. Active ingredients of chemicals are listed in Table 1, footnote b.

Means of the pre-storage treatments after 2 storage periods (summers of 1956, 1957) and 1 growing season (September, 1956, to May, 1957) showed that treatment with Dowicide B resulted in greater bulb yields than did the usual Mersolite W steep. Yields from treatment with Dowicide B were somewhat reduced when the concentration and time were 3 lb. per 100 gal. water for 5 minutes.

Pre-planting treatments did not differ significantly from each other, nor was there a significant interaction between pre-planting and pre-storage treatments, indicating that yields of healthy bulbs primarily reflect degree of control in storage rather than differences that develop during the growing season.

Bulb injury among the various lots treated with Dowicide B varied considerably in both degree and incidence (Fig. 1, Table 3). Lower concentrations and shorter periods produced a slight but conspicuous discoloration; higher rates produced, in addition, a noticeable shrinkage of the inner scales. Mersolite W generally produced less severe injuries. Emmi depressed flower and bulb yields, and a large percentage of flowers were unmarketable because of injury typical of that caused by mercurials. No flower damage was caused by the other treatments.

Bulbs of the Flower Carpet variety were less susceptible to injury than were bulbs of The First

variety: maximum discoloration of the 2 varieties was, respectively, 48.0% and 78.5%. The percentage of bulbs showing injury apparently depended on a number of factors, because percentages varied considerably with a single treatment when the sources of the variety differed.

Since partially cured bulbs of the Helios variety were reported uninjured by Dowicide B (9), fungicidal dips were applied at various times after harvest and related to basal rot and phytotoxicity.

Incidence of injury decreased rapidly as treatment was delayed up to 14 days after harvest (Table 4). Delaying treatment 2–4 weeks reduced bulb discoloration (Fig. 1, Table 4) to slightly under 10% in The First variety, and to about 3% in the King Alfred, a variety that responds to fungicidal dips very much like Flower Carpet.

Bulbs dipped a week or more after harvest developed basal rot in storage as badly as did untreated bulbs. The weight of healthy bulbs, shown in Table 4, is an expression of the incidence of basal rot, which varied from 34% in the control to 9% in the lot treated 1 day after harvest.

DISCUSSION.—These experiments show that narcissus varieties vary considerably in susceptibility to phytotoxicity and basal rot. The First variety is highly susceptible to both, whereas King Alfred and Flower Carpet are more tolerant of *Fusarium oxysporum* f. *narcissi* and not nearly so badly injured by chemical treatment. The very conspicuous discoloration caused by steeping freshly harvested bulbs in Dowicide B solutions would reduce the value of bulbs in the retail trade, where appearance is important. Incidence of injury in The First variety was considerable from treatments applied during the first 7 days after harvesting, and negligible from treatments after longer periods. Injury in the King Alfred variety was significant only if bulbs were treated when freshly harvested. Bulbs grown in soil infested with the soybean cyst nematode should be treated no earlier than 2 weeks after harvest if bulb discoloration is a factor to consider.

TABLE 3.—*The effect of pre-storage fungicidal steeps on discoloration injury to The First narcissus bulbs*

Pre-storage treatment[a]	Discoloration incidence	
	%	Angle[b]
Dowicide B, 6/100, 5 min.	78.5	62.6
Dowicide B, 6/100, 15 min.	72.6	58.4
Dowicide B, 3/100, 15 min.	47.5	43.5
Dowicide B, 3/100, 10 min.	51.8	46.0
Dowicide B, 3/100, 5 min.	53.8	47.2
Emmi, 20 ml/100, 15 min.	57.5	49.4
Mersolite W, 1/500, 10 min.	6.8	13.7
Control	0	0
LSD (.05)		7.3
LSD (.01)		9.9

[a] Unless otherwise noted, concentrations are lb. per 100 gal. water. Active ingredients of chemicals are listed in Table 1, footnote b.

[b] Before statistical analysis, percentages were transformed to angles: angle = arc sin $\sqrt{\text{percentage}}$.

TABLE 4.—*The effect of time of pre-storage treatment[a] on basal rot development and injury in bulbs of The First narcissus*

Days after harvest	Weight of healthy bulbs[b] after storage (g)	Bulbs injured[c]
1 day	1932	44
7 days	1408	20
14 days	1232	6
28 days	1324	8
No treatment	1392	0
LSD (.05)	340	7.6
LSD (.01)	476	10.8

[a] Steeping 15 min. in Dowicide B (85% sodium 2,4,5-trichlorophenoxide) at 3 lb. per 100 gal. water.

[b] Differences in weight are due to incidence of basal rot.

[c] 84 bulbs in each lot.

Effective basal rot control, however, requires treatment as soon after harvest as possible, because basal rot was equally severe when steeping was delayed 7, 14, or 28 days. Bulbs treated about 24 hours after digging showed significantly less rot, indicating that a great many infections develop soon after digging. History of production and preferred disposition of bulbs will have to be considered before the grower can determine the proper treatment to employ.

Although Dowicide B causes conspicuous discoloration and shrinkage of the outer bulb scales, this material proved equal or superior to Mersolite W in pre-storage treatments to control basal rot. In all cases, disease control resulted in increased yields of flowers and bulbs. Pre-storage dips were more effective than pre-planting dips. Of all pre-planting treatments over a 3-year period, Dowicide B resulted in the greatest net gain of healthy bulbs and slabs. Greatest yields of flowers and bulbs almost always occurred when Dowicide B was used at 6 lb. per 100 gal. water for 5 minutes. The ease with which this material goes into solution, plus the short treatment necessary for effective disease control, makes it preferable to Mersolite W where disease control is the major consideration. Mersolite W is very difficult to dissolve in water (6) and requires longer steeping (1) for about the same degree of control.

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EFFECT OF PHOTOPERIOD ON THE MULTIPLICATION OF TURNIP MOSAIC VIRUS IN RAPE[1]

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SUMMARY

When plants of rape were grown under 4-, 8-, 12-, and 16-hour photoperiods, the concentration of turnip mosaic virus and the host growth at 7, 14, 21, and 28 days after inoculation increased with increase in photoperiod.

In 1945, Pound and Walker (6) reported that the concentration of the cabbage A strain of turnip mosaic virus was no different in cabbage grown under a 15-hour photoperiod than in cabbage grown under an 8-hour photoperiod. Their plants were grown in soil, and assays were not made over a period, but at an unstated interval after inoculation. Cheo and Pound (3) found that spinach plants infected with CMV (cucumber mosaic virus) had a higher virus concentration under a 16-hour day than under an 8-hour day, regardless of the interval between inoculation and assay. However, the assay period was only 15 days. In 1956 Pound and Bancroft (5) reported that the concentration of TMV (tobacco mosaic virus) in inoculated leaves of tobacco increased with increased photoperiod at 4 days after inoculation. Systemically infected tissue showed that multiplication of TMV in tobacco plants was initially favored by high light intensity and long day, but both photoperiod and intensity studies showed that the concentration pattern was later reversed, with higher concentrations occurring at low light intensities and short photoperiods. Bancroft (1) recently showed that squash mosaic virus in pumpkin and squash made its greatest initial increase in plants grown at high temperatures (26.5°C) under long days (20 hours), but was ultimately greatest in plants grown at 15.5°C with 8-hour days.

This paper reports studies of the multiplication of turnip mosaic virus in rape (*Brassica napus* L. var. Dwarf Essex) grown under various photoperiods. The purpose was to see if such a virus as turnip mosaic virus, which occurs in the host in comparatively low concentration, differed from TMV in multiplication pattern. Pound and Walker (6) and Pound (4) studied the effect of temperature on the multiplication of turnip mosaic virus in cabbage, but did not make periodic assays over an extended period. They reported that the relationship between temperature and virus concentration was always in the same direction.

MATERIALS AND METHODS.—Rape plants, grown from seed in silica sand in 1-gal. earthenware crocks equipped with automatic siphons, were watered as needed with normal Hoagland's solution. Artificial light banks as described by Pound and Bancroft (5) were 30 in. above the crocks. Photoperiods were terminated by covering crocks with black cages.

The light treatments were started immediately after inoculation. Four crocks containing 5 plants each were used for each treatment in each experiment. Plants were harvested at 7, 14, 21, and 28 days after inoculation, plants in 1 crock being used in each assay. Immediately after harvest, wet weights were taken of whole plants and of leaves only.

Assays of virus concentration in extracts of composite leaf samples were made immediately after harvest by half-leaf inoculations of Havana 38 tobacco plants with the incomplete block design used by Pound (4). Inoculum consisted of sap expressed for 3 minutes in a Waring blendor, filtered, and used at a 1:20 dilution in water. The inoculum was applied uniformly with a 1½-in. nylon brush. Lesion counts were usually made 5–6 days after inoculation. The virus used was the Black Ring (7) strain of turnip mosaic virus.

EXPERIMENTAL RESULTS.—In all series, symptoms appeared about 4 days after inoculation; chlorosis became severe in plants under 4- and 8-hour days. A cyclic development of symptom severity in tip leaves, as described for CMV (3) and TMV (2), was not observed.

Assays from systemically infected leaves showed that virus concentration increased with increased day

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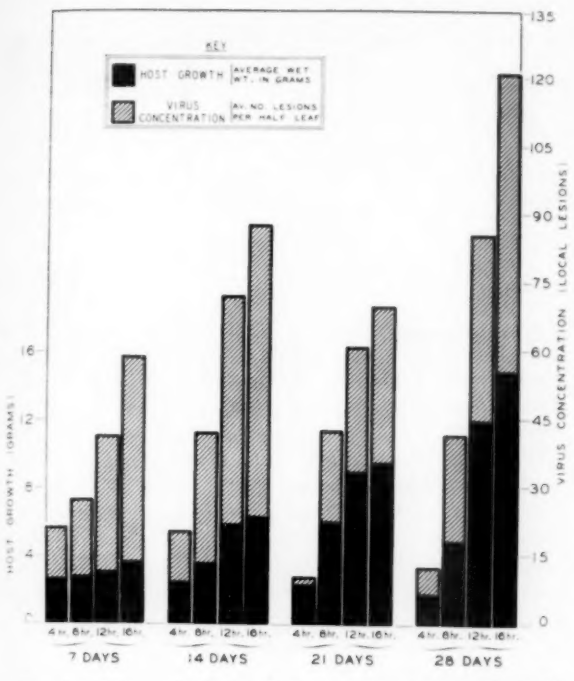

FIG. 1.—Effect of photoperiod on concentration of turnip mosaic virus in rape. Values given are averages of 4 different experiments. Since different assay plants were used in each assay, strict inter-time comparisons cannot be made. Differences between photoperiods were highly significant.

length. In all assays, the greatest virus concentration was found in plants grown under a 16-hour day. As host growth increased, virus concentration also increased (Fig. 1). Largest differences in virus concentrations among treatments occurred in the 28-day assays. In Fig. 1 (in which values are averages of 4 different experiments) virus concentration in relation to host growth is indicated to be lower at 21 days than at 14 days. This is due, not to a drop in virus concentration, but rather to a particular group of assay plants that gave low numbers of local lesions. Other individual assays indicated higher concentrations.

The data in Fig. 1 are only roughly quantitative for virus concentration, but they indicate that, following inoculation, virus synthesis responds more markedly to photoperiod than does host growth. In later assays, virus synthesis more closely paralleled host growth. Critical inter-time comparisons in virus concentrations cannot be made from local lesion data, but it is indicated that, at 12- and 16-hour photoperiods, virus concentration steadily increased throughout the 28 days of the experiments, as did host growth. Under an 8-hour photoperiod, growth increased for a few days and then leveled off. Under a 4-hour photoperiod, virus concentration showed a similar pattern, and there was no indication of increase in either growth or virus concentration after the first assay.

DISCUSSION.—There appear to be 2 distinct responses in plants to photoperiod in regard to virus multiplication. TMV multiplication is initially favored by long photoperiods, but the virus concentration gradient later shifts so that multiplication is greater with shorter photoperiod. Exactly opposite gradients were reported by Pound and Bancroft (5) at 4 and 32 days after inoculation.

With turnip mosaic virus, the relationship between photoperiod and virus concentration is constant: virus concentration consistently increases with increased photoperiod. With this virus there is no evidence of a cyclic concentration pattern in the tip leaves, as is so marked with both CMV and TMV, or in whole plant samples.

In some host-virus combinations, following an initial rapid increase of the virus, the rate of host growth is greater than the rate of virus synthesis (1, 3). Assays made on the basis of host wet weight would thus indicate a decline in virus content. This situation apparently does not exist for turnip mosaic virus in rape or cabbage (4). It is suggested that in these host-virus combinations, virus synthesis and host growth rate are in a balance that is not upset by photoperiod or temperature.

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VARIETAL RESPONSE OF SOYBEANS TO MELOIDOGYNE—A BIOASSAY SYSTEM FOR SEPARATING RACES OF ROOT-KNOT NEMATODES[1]

V. H. Dropkin[2]

SUMMARY

The host-parasite interaction between soybeans and 4 species of *Meloidogyne* are presented as a basis for bioassay procedures to distinguish races of root-knot nematodes. Both galling and egg mass production varied as parasite or host was changed. Egg mass production seemed independent of gall size. The sex ratio varied with different combinations of host and parasite. A California population of *M. incognita acrita* could be distinguished from a Maryland population by differential behavior on soybean varieties.

This investigation was conducted to develop a system for distinguishing populations of root-knot nematodes below the rank of species. Evidence is already at hand on the existence of geographical variants with distinct host ranges within a single species (14, 15). The taxonomy of the genus *Meloidogyne* Goeldi, 1887, rests primarily on the configuration of a cuticular pattern (2); but patterns vary, even among the progeny of single females, and quantitative description of these variations is difficult, making laborious any biometrical morphological approach to the separation of races (6). Host-parasite interaction indicates genetic distinctions among root-knot nematodes more sensitively than does cuticle morphology. A bioassay system could therefore be expected to be useful for studies on heredity in the parasite, factors affecting speciation, questions of geographical distribution, and, most especially, practical determination of the number of races with which the plant breeder must contend. The approach has been to look within single host species for varietal differences in host-parasite interaction with several kinds of *Meloidogyne*. Species studied include *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; *M. arenaria* ssp. *thamesi* Chitwood, 1952; *M. incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. incognita* v. *acrita* Chitwood, 1949; and *M. hapla* Chitwood, 1949.

This report includes the results of trials with 9 inbred lines of corn (*Zea mays* L.), 10 lines of oats (*Avena* sp.) and 19 varieties of soybeans (*Glycine max* (L.) Merr.). Studies were carried out on giant cell formation in susceptible and resistant varieties of soybeans, the parasite's sex ratio was measured in various hosts, and a system was developed for collecting larvae in large numbers.

MATERIALS AND METHODS.—For each of the 3 host plant species, inoculum consisted of soil and roots from greenhouse populations of single species of *Meloidogyne*. Seeds of test varieties were planted in 2–4 replications in 4-in. pots filled with thoroughly mixed inoculum. Roots were washed and scored at appropriate times. In the soybean trials, each host-nematode combination was repeated 4 times; after 51 days, roots (placed by an assistant into coded Petri dishes for examination without magnification) were separated into 4 classes according to degree of galling. Egg mass production was evaluated under 30× magnification, in 3 classes according to number and 3 classes according to size. Histological preparations of soybean roots were made by standard methods of paraffin embedding after fixation in Randolph's modification of Navashin's fixative; sections were cut at 10 μ and stained with safranin-fast green. Sex ratios were estimated from counts of root samples carried out until 200 adult females were totaled per soybean variety.

Larvae were collected from heavily infected tomato and soybean plants that had been held at wilting point for 2 weeks before collection. Roots were carefully washed free of soil, cut into ½-in. lengths, and placed on wire mesh in Petri dishes. Just enough water was added to wet but not cover the roots (11). Larvae emerged at a rate of 20,000 or more per plant per day, totaling 100,000–200,000 per plant. The 2-week period at wilting point prevented larvae from emerging but did not prevent normal development, so that larvae emerged in great numbers from roots subsequently exposed to water (9).

RESULTS.—*Corn.*—Nine inbred lines of corn[3] were exposed for 7–21-week periods to 5 species of *Meloidogyne* in greenhouse tests. Each line responded in an individual manner to the nematodes, and each nematode species responded in an individual manner to the inbred lines. Table 1 summarizes the ability of each host to maintain infection. No lines were encountered that were poor hosts for all types of *Meloidogyne* tested, but such lines probably exist. Differences appeared in the size and number of galls. Line A-73 had conspicuous confluent galls with *M. incognita* but much smaller galls with *M. incognita acrita*. Egg masses were large in both cases. Line 38-11 with *acrita* was characterized by large galls with poor egg masses, whereas Oh-51 with *acrita* displayed small galls with large egg masses. In some

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[3] Seed was obtained through the cooperation of Dr. H. T. Stinson, Connecticut Agricultural Experiment Station, New Haven.

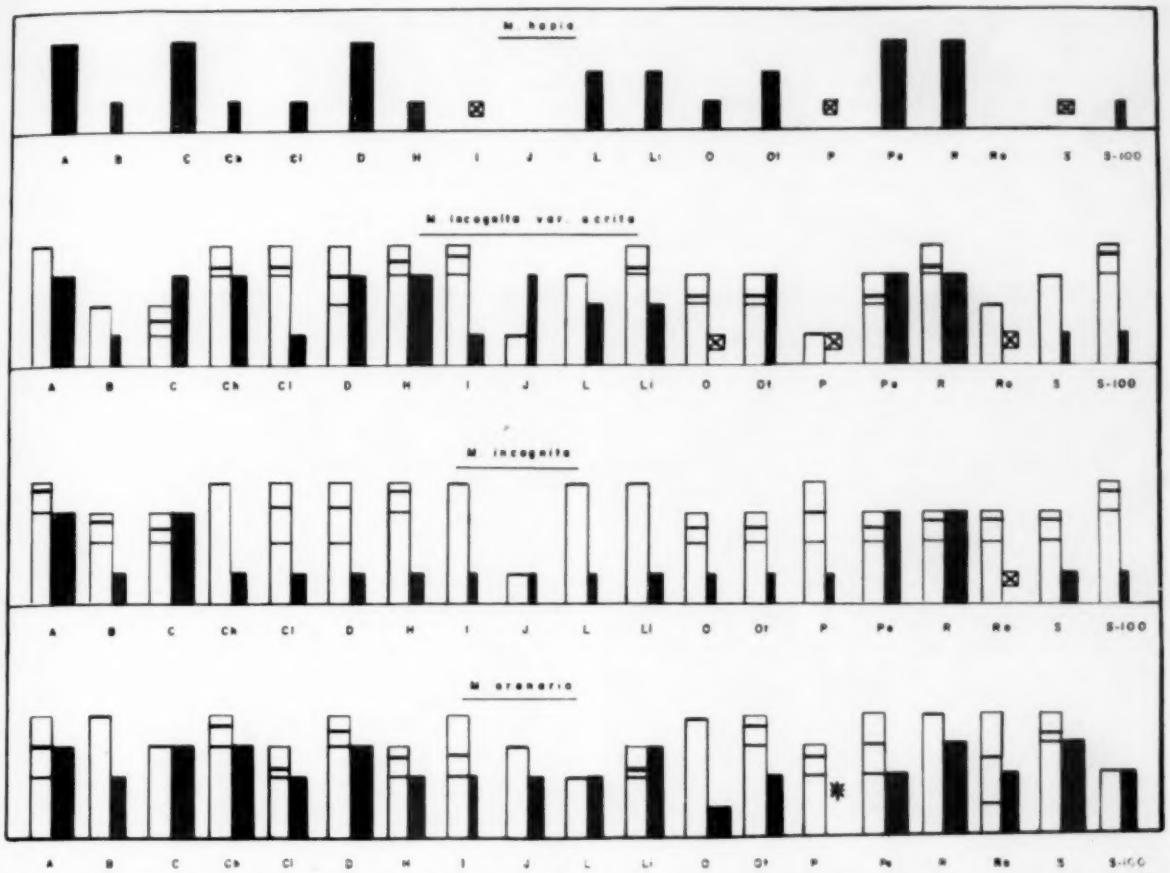

FIG. 1. Amount of galling and egg mass production in 19 varieties of soybeans exposed to 4 populations of *Meloidogyne*. Height of white bars indicates amount of galling, with mean and range. Height of shaded bars shows number of egg masses, and width shows size. X in box signifies no egg masses. * indicates variable egg masses. A = Adams variety, B = Blackhawk, C = Capitol, Ch = Chief, Cl = Clark, D = Dorman, H = Harosoy, I = Improved Pelican, J = Jackson, L = Lee, Li = Lincoln, O = Ogden, Ot = Ototan, P = Palmetto, Pe = Perry, R = Richland, Ro = Roanoke, S = Seminole, S-100 = S-100.

combinations brownish discolorations within roots were commonly present near nematodes. Several instances of purple discoloration were seen around the parasites, and in one case the nematodes were blackened.

Soybean.—1) *Galls and egg masses.*—Although corn offered ample evidence of the genetic variability of host response to root-knot nematodes, I felt that a search for a more suitable host was warranted to find greenhouse plants that show greater variability in response to *Meloidogyne*. Ten inbred lines of oats proved even less promising than corn, because galls were small and roots became matted.

Soybeans[4] proved highly suitable for bioassay work. Many varieties exist, the range of response to infection is very great, reproduction is by self-pollination, and greenhouse management is convenient. Results of variety tests are presented in Fig. 1. Inoculum was carefully mixed for each series so that exposure to infection was comparable from plant to plant within a series, though not between series. Gall scores were therefore estimated independently for each series. In the *M. incognita* and *M. incognita acrita* infections, galls were well distributed over the entire root system and ranged from massive to very slight. In *M. arenaria* infections, however, galls were largely confined to the tap root; the massive swellings produced with *M. incognita* and *M. incognita acrita* were absent. In *M. hapla* infections, individual galls were too small to score the roots. Typical root symptoms are shown in Fig. 2.

The results of the soybean tests may be summarized as follows:

A) Soybean varieties exposed to a single population of root-knot nematodes differed both in galling and in the success of the nematode (egg mass production). In the *M. incognita acrita* series, 4 varieties were lightly galled, 10 were heavily galled, and 5 were intermediate. Egg mass production ranged from high (in 7 varieties) through intermediate (in 4) to low or absent (in 8).

[4] Seed was obtained through the cooperation of Dr. H. W. Johnson, Soybean Section, Crops Research Division, U. S. D. A., Beltsville.

B) A single variety exposed to different populations of nematodes showed varied responses in galling and egg mass production. Variety Jackson appeared moderately galled by *M. arenaria* but almost free of effects from *M. incognita* or *M. incognita acrita*. Lee showed marked galling with *M. incognita* but slight galling with *M. arenaria*. All varieties had small galls with *M. hapla*. Harosoy displayed abundant egg masses with *M. arenaria* but none with *M. hapla*.

C) Egg mass production is the most sensitive indicator of the parasite's welfare. Plants were lifted when egg masses were well developed on Richland, which our tests had shown to be highly susceptible to all nematodes tested. Varieties in which nematodes developed abundant large egg masses included both heavily galled (Harosoy, Adams, and Richland with *M. incognita*) as well as lightly galled varieties (5 lines with *M. hapla*, and Capitol with *M. incognita*

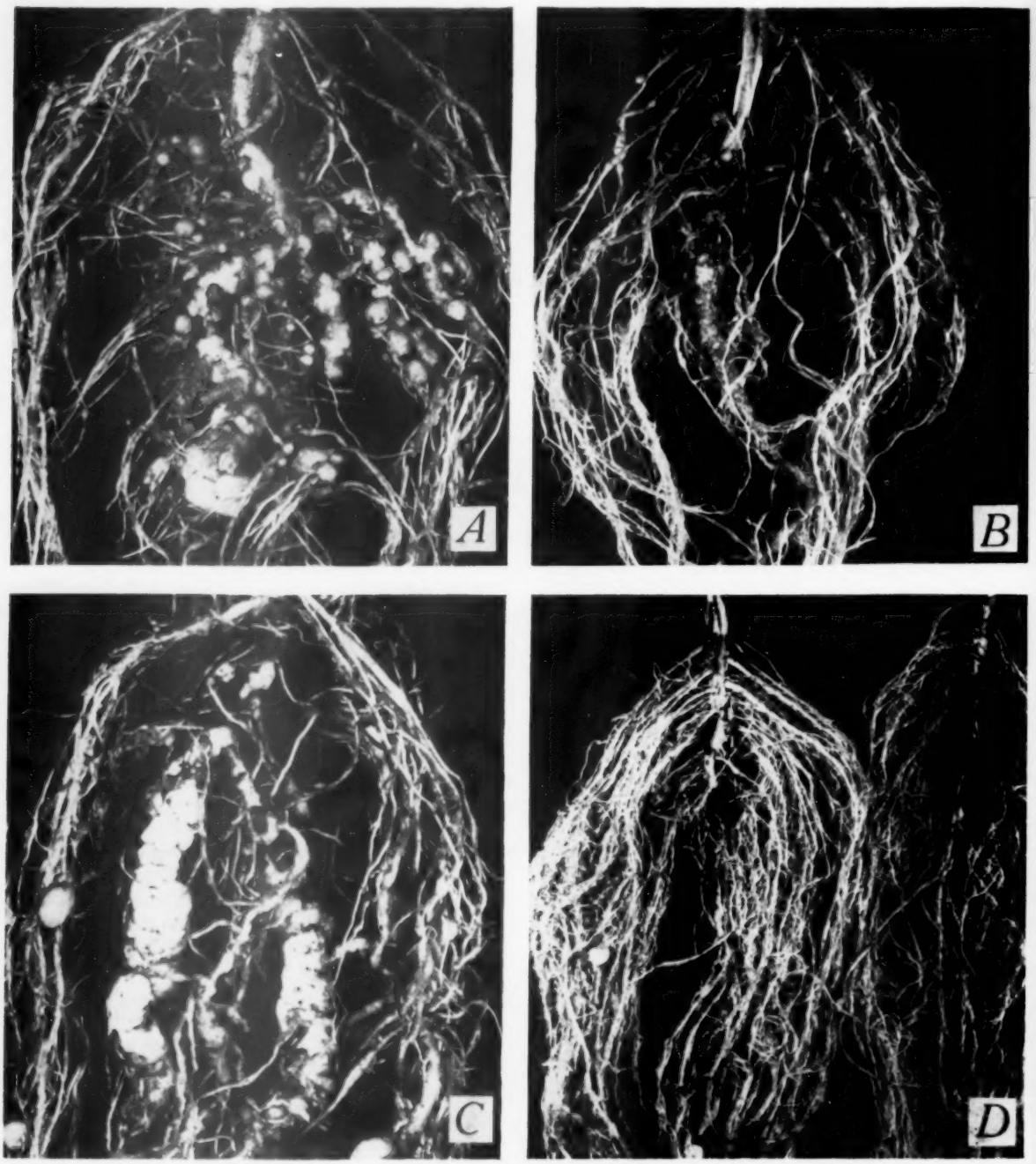

FIG. 2. Galling response of soybean varieties to infection with *Meloidogyne* spp. A) Variety S-100 with *M. incognita acrita*; B) S-100 with *M. arenaria*; C) Lincoln with *M. incognita*; D) Jackson with *M. incognita*.

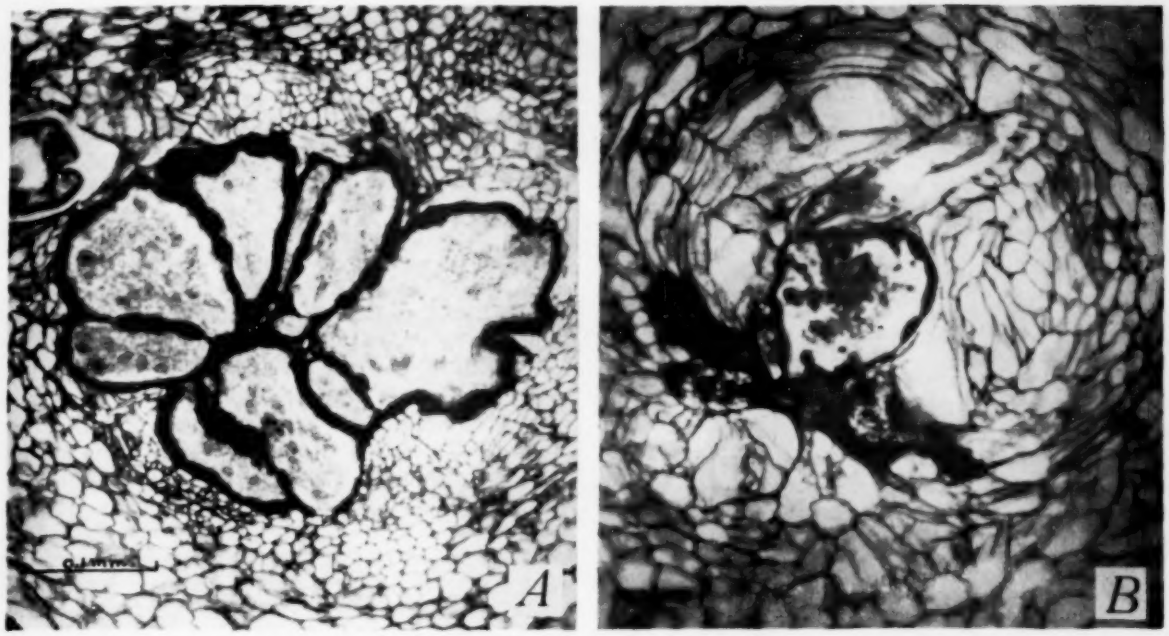

FIG. 3. Giant cells in soybean varieties infected with one female of *Meloidogyne incognita*. A) Variety Richland; B) variety Seminole.

acrita). Poor egg masses were found in both heavily and lightly galled varieties.

One example of the use of the soybean variety test to separate populations within a single species can be presented. Population L-3 (identified as *M. incognita acrita*) originated in California.[5] The population of *M. incognita acrita* shown in Fig. 1 originated in Maryland. Varieties Dorman, Lee, Improved Pelican, Richland, and Seminole responded to both populations in the same way. With L-3, Harosoy bore very few egg masses, and Roanoke had moderately abundant egg masses, whereas with the Maryland population Harosoy produced abundant egg masses, and Roanoke none at all. The L-3 population from California was differentiated from the Maryland population of *M. incognita acrita* by its behavior on these 2 varieties of soybeans.

2) *Giant cells.*—Histological sections revealed a correlation between giant cell structure and egg mass production as judged externally. Six varieties were studied in combination with *M. incognita* and *M. incognita acrita*. Giant cells in the highly susceptible Richland were large and smooth, with uniformly stained contents and thick walls. They resembled giant cells recently described in sweet potato (13). Dorman had such cells in some clusters, as well as much smaller, irregular giant cells with strands of deeply staining cytoplasm and relatively thin walls. Seminole in both combinations and Lee with *M. incognita* were characterized by small giant cells, whereas Lee with *M. incognita acrita* resembled the Richland series. Some evidence of cell necrosis was present around the nematodes in Seminole, Lee, and Improved Pelican in both combinations. Fig. 3A, a section from Richland with *M. incognita*, shows the maximum diameter of giant cells associated with the head of a single female nematode. Fig. 3B is a corresponding section from Seminole with *M. incognita*. The contrast between these suggests that resistance is related to the cellular response of the host to the parasite. More detailed studies on giant cell formation in a resistant and susceptible soybean are under way.

3) *Sex ratios.*—Another striking varietal difference appeared in the relative numbers of males and females in roots at fixation (51 days after exposure). Four samples of large galls and 4 of small galls were dissected for each of 19 varieties infected with *M. incognita* and each of 5 varieties infected with *M. arenaria* and *M. incognita acrita*. Male abundance could not be related to size of gall. No clear relation could be seen between success of the parasite and male abundance, for although varieties with abundant egg masses had few males within the roots, poor hosts varied from low to high numbers of males. Since it is possible that males had been present earlier but had left the roots before fixation, these data may be interpreted as showing differences in developmental rates. They also offer the strong probability that real differences occur between varieties in parasite sex ratios. Great differences in numbers of males were found among 5 varieties with 3 kinds of nematodes. *M. incognita* averaged 46% males, *M. incognita acrita* averaged 19% males, and *M. arenaria* had 9% males.

DISCUSSION.—Bensley (1) has stated the case for a

[5] Obtained through the cooperation of Dr. L. A. Lider, Department of Viticulture, University of California, Davis.

TABLE 1.—*Reproduction of Meloidogyne spp. on lines of inbred corn*

	Meloidogyne spp.				
Corn line	*arenaria*	*arenaria thamesi*	*incognita acrita*	*incognita*	*hapla*
M-14	+	+	+	+	
Os-420	0	0	+	0	0
W-F-9	?	0	?	+	0
C-103	?	0	+	+	
A-73	?	0	+	+	
Oh-51	?	+	?		
W-22	?	0	+	+	0
38-11	?	+	0	?	0
B-14	+	+	+	+	0

+ = abundant reproduction; 0 = no reproduction; ? = minimal reproduction; blank indicates no test.

bioassay approach: "In our enthusiasm for these methods (of microchemistry), however, we must not forget that by far the most sensitive instrument for microchemical analysis is the living organism itself. The methods of immunology, for example, suffice to discriminate between compounds so closely related that the chemist is at a loss to distinguish between them. The genes revealed by genetic experiment exceed by an infinite multiple the meager number of nucleoproteins revealed by biochemical research. The bioassay methods depend on the exquisite sensitivity of the living organism to minute changes in its chemical environment. These are also microchemical methods."

That soybeans and root-knot nematodes show "exquisite sensitivity" to each other is demonstrated in the present work. Most of the relationships found were described earlier by Christie, in papers on host-parasite relationships that formed the foundation for subsequent taxonomic clarification of the genus (2, 3, 4, 5). Methods used in the present and all previous studies were crude; the next obvious task is to refine for control of the many variables that may affect phenotypic expression of host-parasite interactions. For example, number of larvae and time of infection undoubtedly influence degree of galling. A set of trials to determine a proper level of inoculum showed that 10,000 larvae in a 4-in. pot sufficed to stop the growth of susceptible varieties sown from seed, but had less effect on seedlings exposed at the 3-leaf stage. In the same way, it is necessary to test the effect of temperature, nutrition, light, moisture, and other environmental conditions on host-parasite interaction.

The results obtained raise many puzzling questions. Infections with *M. arenaria* were largely confined to the tap root, but infections with other populations were more uniformly distributed over the entire root system. Does this indicate a differential attractiveness of the tap root to larvae of *M. arenaria* but not to other species? Previous experience with both *Meloidogyne* on cucumber (7) and *Heterodera* on tomato (8) indicates that each larva enters a root independently. This may be untrue in soybean roots infected with *M. arenaria*, so that the tap root drains off most of the available larvae. The question of sex determination is another interesting puzzle. A correlation between nutrition and sex determination is suggested in the literature (10, 16). Golden and Schafer (12) reported variations in sex ratios of *Heterodera schachtii* Schmidt in selections of *Chenopodium album* L. that are similar to the results with soybeans. According to the nutrition theory, poor hosts are presumably poor sources of nutrition of the parasites and thus should favor the development of males. Similarly, large galls containing many larvae should be more favorable to male development than small galls infected with one or a few nematodes. The data show low numbers of males in adequate hosts, as expected, but some poor hosts had high proportions of males and others had very few. Furthermore, over 90% of the males were in large galls in some combinations, and over 75% of the males were in small galls in other combinations. This suggests a more complex situation, and indicates that specific conditions present in some hosts influence male development.

Christie (4) suggested that there are several bases for host resistance to root-knot, including lack of larval entry, failure to respond to larval secretions with giant cell formation, and root tissue necrosis during early stages of parasitism. Studies of the infectivity of larvae to soybean varieties will undoubtedly show differences among varieties in the initial success of penetration. Both giant cell size and character vary among different hosts exposed to the same parasite. Similar variations occur when one host is exposed to different parasites. Tissue necrosis likewise characterized some host-parasite combinations but was absent in others. Soybean varieties, therefore, offer excellent material for study of the nature of resistance to root-knot. The great sensitivity of soybean varieties to differences among these parasitic nematodes combines with the sensitivity of the nematodes to differences among hosts to make soybeans excellent tools for the separation of genetically distinct parasite populations.

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INFLUENCE OF SOIL TEMPERATURE AND OF STRAINS OF THE PATHOGEN ON SEVERITY OF VERTICILLIUM WILT OF PEPPER[1]

J. B. Kendrick, Jr., and John T. Middleton

SUMMARY

Pathogenicity of microsclerotia-producing isolates of *Verticillium albo-atrum* Reinke & Berth. obtained from boysenberry, icicle radish, chrysanthemum, strawberry, potato, stock, rose, avocado, muskmelon, okra, tomato, and pepper plants was compared in hot and sweet pepper varieties, using a stunting index to assess disease severity. The isolate from pepper differed outstandingly from all others in that it alone produced severe stunting and disease development in the pepper varieties studied. Isolates from chrysanthemum and rose induced mild stunting; all others were nonpathogenic to pepper. Although the pepper isolate affected okra severely, it failed to establish itself in a susceptible tomato host. Maximum growth rate of *V. albo-atrum* from pepper occurred at 24°C, but good growth developed between 12° and 30°C. Maximum growth rate of 6 pepper varieties studied at an air temperature of 24°C occurred with a soil temperature of 30°C, although 25°C was almost as favorable. The disease at 24°C air temperature was severe at 15–30°C soil temperatures, but decreased markedly at 35°C. Severity of disease expression seems, therefore, more closely associated with growth of the pepper pathogen than with growth of its host. The pepper varieties used in these studies, although differing slightly in susceptibility to infection and disease development induced by the pepper isolate, did not seem to have any substantial resistance to the disease.

Verticillium wilt of pepper, *Capsicum annuum* L., incited by *Verticillium albo-atrum* Reinke & Berth., sometimes reaches epidemic proportions in certain fields in southern California. The widespread distribution of this fungus in southern California would lead one to expect Verticillium wilt of pepper to occur more frequently than it actually does. Although there are conflicting reports in the literature on the pathogenicity of *V. albo-atrum* to pepper, Snyder and Rudolph (12) presented conclusive evidence that this organism is a true parasite of pepper. Haenseler (4) reported unsuccessful attempts to infect pepper with an isolate of *V. albo-atrum* that produced severe disease symptoms on eggplant. Later, however, he listed pepper as a host for this organism without indicating why he had revised his opinion (5). Ludbrook (9), using a number of isolates of *V. dahliae* Klebahn and *V. albo-atrum* from different hosts, was unable to demonstrate their pathogenicity in pepper. Bewley (1), on the other hand, reported that *V. albo-atrum* obtained originally from tomato was pathogenic to pepper, although the disease was not so severe as on tomato, cucumber, and certain other hosts. Ludbrook (9) likewise reported that *V. dahliae* was less pathogenic than *V. albo-atrum* to tomato, potato, and cucumber, but that some isolates of *V. dahliae* were more pathogenic to tomato than were cultures of *V. albo-atrum*. Nelson (10) reported that *V. albo-atrum* isolates from hosts other than *Mentha piperita* L. were not pathogenic to Mentha, although the isolates from wilting peppermint and spearmint were pathogenic only to menthaceous plants and *Monarda* spp. Evidence presented below suggests that pathogenically distinct strains of *V. albo-atrum* may also exist with respect to pepper.

The influence of soil temperature on the development of Verticillium diseases of herbaceous crops has been reported by several workers. Nelson (10), Bewley (1), Chaudhuri (2), Ludbrook (9), and Edgington and Walker (3) found that the optimum temperature for growth of Verticillium in culture is near 23°C.

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Paper No. 1048, University of California Citrus Experiment Station, Riverside, California.

There is less agreement as to the optimum temperature for disease development, as might be expected when one considers the simultaneous action of temperature on both host and parasite growth. Bewley (1) reported that Verticillium wilt of tomato was most severe at 16–20°C, with 25°C the upper limit for disease development. But he used averages of maximum and minimum temperatures, which probably gave a false impression of the critical relationship of the cardinal temperatures. Roberts (11) reported that 23.4°C was optimum for development of Verticillium wilt of tomato. Ludbrook (9) stated that *V. dahliae* produced severe symptoms in eggplant at 12–30°C, whereas *V. albo-atrum* produced similar symptoms in the same host at 12–28°C. The maximum temperatures for infection by the 2 organisms were 32° and 30°C, respectively. Ludbrook reported that *V. albo-atrum* incited maximum disease development in tomato at 24°C, with 28°C the upper limit for disease expression. Nelson (10) noted severe wilt of mint at 24° and 28°C. Edgington and Walker (3) reported severe disease development in tomato at soil temperatures of 20° and 24°C with air temperatures of 16°, 20°, and 24°C, for both microsclerotial and non-microsclerotial strains of *V. albo-atrum;* but only the microsclerotial strain produced severe symptoms at soil and air temperatures of 28°C.

The soil temperatures recorded for various Verticillium wilt diseases range from 12° to 30°C. The effect of soil temperatures on Verticillium wilt of pepper is discussed in this paper, as is varietal susceptibility of the pepper host. Both factors may be of importance in explaining the irregular occurrence of the disease in California.

MATERIALS AND METHODS.—One of the most characteristic symptoms of Verticillium wilt of pepper is stunting, an effect often noted even before the onset of yellowing and inward rolling of leaves. In later stages of the disease, leaves wither and drop. Stunting results from cessation of elongation of internodes still in a developmental stage. The use of stunting to rate disease severity has been described by Kendrick and Middleton (6, 7). Stunting index is a ratio of plant growth between uninoculated control plants and infected plants growing under the same environmental conditions.

Preliminary tests showed 4 weeks to be the minimum time from inoculation to pronounced disease manifestation. In these studies the ratio of growth between control and diseased plants is calculated from 4-week-growth increments. At the time of inoculation, individual control and inoculated plants were measured from the point of the first leaf attachment to the growing tip; 4 weeks after inoculation the same plants were again measured from the same points of reference. An average 4-week-growth increment calculated for the control plants was used as the dividend; the 4-week-growth increment of the individual inoculated plant was used as the divisor. The quotient was the stunting index. When it seemed advisable, individual stunting indexes for a given treatment were averaged and treated statistically.

When this method of calculation is applied to uninoculated control plants, the value derived is about 1.0. Thus the base value for comparing disease effect is near 1.0. For example, any group of similarly treated plants that have an average rating near 1.0 would have a growth about the same as that of the control plants. The greater the index, the more severe the stunting symptom.

Preliminary studies were also made to determine optimum seedling age for inoculating to obtain pronounced expression of the stunting symptom. Plants inoculated 30 days from seeding were compared with plants inoculated 60 days from seeding. Both sets of plants, 2 weeks after seeding, had been transplanted

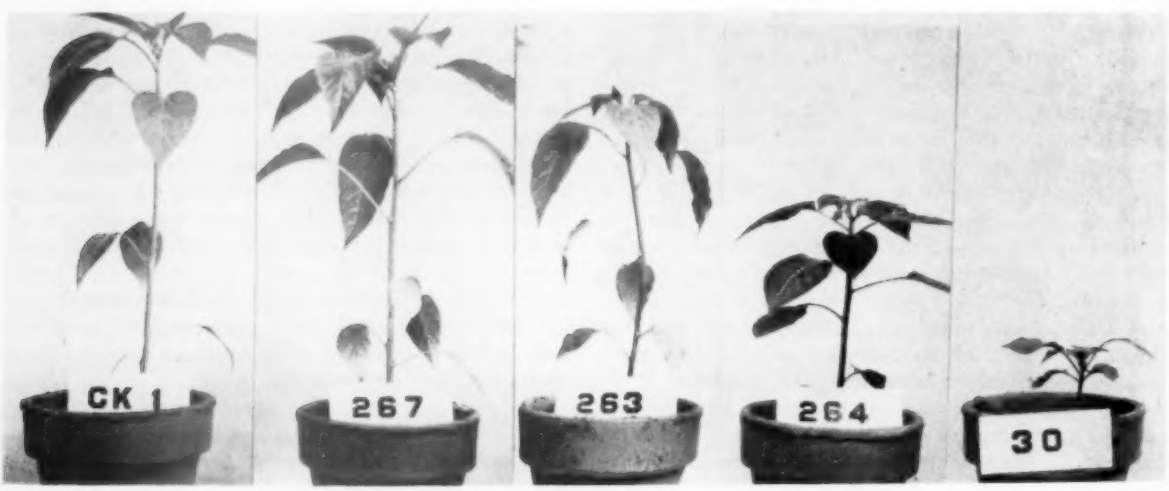

FIG. 1. Relative stunting of Anaheim Chili pepper plants 4 weeks after inoculation with isolates of *Verticillium albo-atrum* from various hosts: CK 1, uninoculated control; No. 267, isolate from stock; No. 263, isolate from boysenberry; No. 264, isolate from chrysanthemum; and No. 30, isolate from pepper. All plants 58 days from seeding.

into flats of 50 plants each. At inoculation time the 30-day plants had 2 fully expanded and 2 developing leaves; the 60-day plants had 10 fully expanded and 3–4 developing leaves. Under optimum conditions for disease development the 30-day plants had a stunting index about 15 times that of the 60-day plants under the same conditions. Therefore, 30-day pepper plants were used for inoculations in all experiments reported herein.

As inoculum sources, spores alone were compared with mycelial fragments plus spores obtained from a 23-day-old potato-dextrose agar culture. The inocula were suspended in distilled water and diluted to the point where, in one case, 50 spores, and in the other case, fragments plus spores equalling 50, were counted in a microscope field at 440×. Since there was no significant difference between the 2 types of inocula in degree of disease severity as expressed by stunting index, spore suspensions (50/440×) were used as the standard inoculum.

Plants were inoculated by removing them from soil, washing off the soil particles clinging to the roots, and immersing the roots 1 minute in the standard spore suspension. The plants were then reset in soil, watered, and placed in the environments being studied. Control plants were treated in like manner except that the roots were dipped into distilled water instead of spore suspensions.

RESULTS.—*Influence of fungus strain on disease severity.*—To test the possibility that strains of *Verticillium albo-atrum* might differ in pathogenicity to pepper, isolates were obtained from a wide variety of hosts. Dr. Stephen Wilhelm, of the Department of Plant Pathology, University of California, Berkeley, kindly supplied isolates from boysenberry, icicle radish, chrysanthemum, strawberry, potato, stock, and rose. The authors obtained additional isolates from avocado, muskmelon, okra, and tomato. All hosts yielding the different isolates were from various parts of California. The pepper isolate was obtained from severely affected plants growing in the coastal regions of southern California. All isolates were microsclerotia producers and indistinguishable in cultural characteristics.

In one test, 3 isolates from boysenberry and 1 each from icicle radish, chrysanthemum, strawberry, potato, stock, rose, and pepper were compared for pathogenicity to chili pepper (variety Anaheim Chili). Only the isolates from chrysanthemum, with stunting index 2.2; rose, with 2.3; and pepper, with 7.7, were significantly pathogenic to pepper. The pepper isolate is in a class by itself in pathogenicity to this host, because the difference in disease severity between this and all other isolates and the uninoculated control is highly significant. This difference in disease incitement among the different isolates of *V. albo-atrum* is shown clearly by representative isolates in Fig. 1.

Two varieties of chili pepper (Anaheim Chili and Jalapeno) and 1 variety of sweet pepper (California Wonder) were used to rate the relative pathogenicity of 5 different isolates of *Verticillium albo-atrum* cultured from avocado, muskmelon, okra, tomato, and pepper. Table 1 shows the results of one of these tests.

TABLE 1.—*Mean stunting indexes of Verticillium wilt expression in Anaheim Chili, Jalapeno, and California Wonder peppers inoculated with Verticillium albo-atrum from different hosts*

Isolate number	Original host	Stunting index for pepper varieties: Anaheim Chili	Jalapeno	California Wonder
30	Pepper	5.7	4.3	3.7
94	Avocado	1.0	1.3	1.1
98	Muskmelon	1.1	1.2	1.3
231	Okra	1.3	1.3	1.3
269	Tomato	1.2	1.3	1.1
Uninoculated		1.1	1.1	1.1
LSD	19:1	0.4	0.4	0.2
LSD	99:1	0.5	0.5	0.3

Again, the pepper isolate, No. 30, was in a class by itself in pathogenicity to pepper. It was severely pathogenic to the 3 different varieties of pepper, but to different degrees, as exemplified by the range of values of the stunting indexes. The California Wonder variety was not as severely stunted by the pepper isolate as were the other 2 varieties, but it was the only pepper variety significantly stunted by isolates No. 98, from muskmelon, and No. 231, from okra. Both of these isolates were less pathogenic to this variety, however, than was the pepper isolate.

Pepper isolate No. 30 produced severe stunting and subsequent death of okra. It failed to establish itself in tomatoes (variety Pearson) with standard inoculation used in these studies. In this test, pepper plants (variety Jalapeno) inoculated with isolate No. 30 were severely stunted and diseased.

Influence of temperature upon parasite, host, and disease development.—Four different isolates, representing a range of pathogenicity to pepper displayed by those in this study, were used to examine the influence of temperature on growth rate: No. 30, from pepper; No. 94, from avocado; No. 231, from okra; and No. 269, from tomato.

Growth studies were made in test tubes 2.5 cm in diameter and 18.5 cm long, with a total capacity of about 70 ml. A depression was made in the glass 5 cm from the mouth, forming a glass dam that prevented the liquid agar from flowing out when the tube was placed flat on a table. Ten ml of potato-dextrose agar was added to each tube; the medium was autoclaved and allowed to cool and solidify in a horizontal position so that a uniform thickness of agar was formed with a flat surface about 130 mm long. The isolates were initially grown on potato-dextrose agar in standard Petri plates until the plates were about ⅔ filled. Then, disks 5 mm in diameter were cut from the margin of the culture, and one disk was placed on the agar surface close to the glass dam in

TABLE 2.—*Growth of several pepper varieties as influenced by soil temperature, expressed as mean daily-increment stem elongation*

Variety	Mean daily-increment (mm) at soil temperatures indicated: 15°C	20°C	25°C	30°C	35°C
Anaheim Chili	0.7	1.6	2.5	2.9	2.0
Jalapeno	0.4	2.0	2.6	3.6	2.3
Floral Gem	0.9	2.2	3.0	3.0	2.6
Pimiento Perfection	0.7	1.4	1.9	2.4	1.6
Hungarian Paprika	0.5	1.4	2.3	2.5	1.2
California Wonder	0.4	1.2	2.2	2.6	1.3
All varieties	0.6	1.6	2.4	2.8	1.8

each tube. Four tubes were prepared and used at each of the temperatures in the study. The minimum temperature was 3°C; additional studies were made at 3-degree intervals to a maximum of 39°C. Growth from the edge of the disk to the advancing margin of the mycelium was measured daily for 7 days. An average daily growth-increment in mm was calculated for each of the isolates at each of the 13 temperatures.

The maximum daily growth-increments (mm) for isolates 30, 94, 231, and 269 were 1.6, 1.8, 2.1, and 1.9, respectively. These all occurred at 24°C, which, for this pathogen, is in substantial agreement with reports by most other investigators. No growth was evident after 7 days for any isolate below 9 or above 36°C. The pepper isolate grew at a slightly slower rate than the other 3 at all temperatures where growth occurred, although each grew reasonably well over a temperature range of 12–30°C.

Six different varieties of pepper were used to evaluate the influence of soil temperature on vegetative growth. Hot, or chili, pepper types were represented by the varieties Anaheim Chili, Jalapeno, and Floral Gem; pimiento by Pimiento Perfection; paprika by Hungarian Paprika; and sweet pepper by California Wonder. Plants were transplanted 2 weeks after seeding into galvanized cans containing chloropicrin-treated soil. When the plants had resumed normal growth, the galvanized containers, each with 15 seedlings, were immersed to the soil line in water tanks maintained at 15°, 20°, 25°, 30°, and 35°C. The air temperature during these experiments remained near 24°C.

Table 2 shows the influence of soil temperature on the growth of the different varieties, expressed as the mean daily-increment stem elongation. Maximum growth rates occurred at 30°C for all varieties, but in other temperature relations, varieties differed. The growth rate of Floral Gem was not greatly altered at 20–35°C, whereas that of Jalapeno was strongly influenced by temperature changes in the same range. Another difference was that the 3 hot varieties tolerated 35°C soil temperature better than the other varieties used in this test.

Soil temperature effects on Verticillium wilt expression in peppers were studied in the same manner and at the same time as their effects on plant growth *per se*. Plants were inoculated and stunting indexes calculated as described above. The results of typical experiments are presented in Table 3.

These data show that, as far as Anaheim Chili, Jalapeno, and California Wonder varieties are concerned, disease severity is similar at soil temperatures of 15–30°C. Although Floral Gem, Pimiento Perfection, and Hungarian Paprika appear less severely diseased at 15° than at 20°, 25°, or 30°C, only Pimiento Perfection reacted in this manner consistently in other tests. Therefore, it is quite probable that no significant difference in disease severity exists between 15° and 30°C for all varieties tested, with the possible exception of Pimiento Perfection.

At 35°C, symptoms of Verticillium wilt were very mild in Anaheim Chili and Hungarian Paprika, and almost completely absent from the other varieties. In every case stunting indexes of infected plants grown at soil temperatures of 35°C were reduced significantly below those of plants grown at 30°C.

In comparing varieties only, Anaheim Chili was stunted most severely, followed in order by Hungarian Paprika, California Wonder, Floral Gem, Jalapeno, and Pimiento Perfection.

The relation of soil temperature to host, pathogen, and disease development, and the influence of relative growth of host and pathogen on disease severity, is graphically shown in Fig. 2. The curve for host growth is derived from the mean daily-increment growth values for all varieties listed in Table 2 at each of the 5 soil temperatures. The curve for stunting index is constructed from the mean values, shown at the bottom of Table 3, for all varieties. The curve for pathogen growth represents mean values secured from 4 tests using pepper isolate No. 30. The ratio

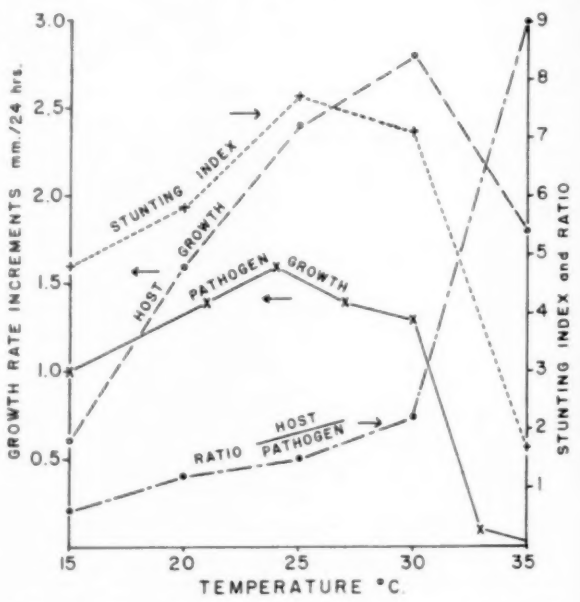

FIG. 2. The relation of soil temperature to host, pathogen, and disease development, and the influence of relative growth of host and pathogen on disease severity.

TABLE 3.—*Verticillium wilt stunting indexes of 6 pepper varieties as influenced by various soil temperatures*

Variety	Stunting indexes at soil temperatures indicated:					LSD	
	15°C	20°C	25°C	30°C	35°C	19:1	99:1
Anaheim Chili	12.2	7.6	12.4	11.7	3.0	6.6	8.7
Jalapeno	4.5	4.6	4.3	5.0	1.1	2.6	3.5
Floral Gem	1.9	8.4	6.4	6.1	0.8	2.3	3.0
Pimiento Perfection	1.2	2.9	4.9	5.8	1.2	1.6	2.2
Hungarian Paprika	3.0	5.8	10.6	8.6	2.6	4.0	5.3
California Wonder	6.0	5.3	7.6	5.4	1.3	2.3	3.0
All varieties	4.8	5.8	7.7	7.1	1.7		

of host growth to pathogen growth was calculated by dividing the combined mean-increment-growth values for the 6 varieties by the mean-increment-growth values of Verticillium isolate No. 30 for each soil temperature studied.

It will be noted that the curves for pathogen growth and stunting index are almost parallel and follow the same pattern. The curves for host growth and host-pathogen ratio do not, however, appear to have any relationship to the stunting index.

DISCUSSION.—These studies demonstrate that pathogenically different races exist within *Verticillium albo-atrum*. A significantly greater disease expression was induced in pepper plants by an isolate of *V. albo-atrum* originally from diseased pepper than by isolates from other hosts. Nelson (10) reported a comparable observation for a race highly pathogenic to *Mentha* spp. In these studies no attempt was made to define races rigidly by determining the pathogenic capabilities of the isolates in all possible combinations of cross inoculations. However, when pepper isolate No. 30 was used to inoculate okra and tomato it was found that severe disease expression developed only in the former. This leads one to assume that, although pathogenic specificity may exist in *V. albo-atrum*, it probably has broader host limits than have some of the Fusarium wilt-inducing pathogens.

Variation in host susceptibility to Verticillium wilt must not be overlooked in considerations of pathogenesis of the Verticillium isolates. It is possible that certain hosts—okra, for instance—might react with great sensitivity to very small amounts of, or even chemically different, toxins produced by different races or isolates of *V. albo-atrum*. Other hosts, such as pepper, conceivably could tolerate all but high concentrations of the wilt-inducing toxin or most of a group of chemically different Verticillium toxins, if they exist. It is not now possible to state whether pathogenically specific races of *V. albo-atrum* exist because pathogenesis is peculiar to a given race, or whether disease expression differs only in levels of toxin production from different isolates, or whether disease differences result from varying host tolerances to toxin concentration.

Regardless of the reason for differences in disease severity, the existence of isolates of *V. albo-atrum* with different pathogenicities is evidenced by the results reported. This fact explains in some measure why Verticillium wilt of pepper is not as widespread or as serious a disease as one might expect it to be on the basis of the distribution of the pathogen in southern California, and is a possible explanation for the earlier conflicting reports of Haenseler (4, 5), Ludbrook (9), and Bewley (1) concerning the pathogenicity of *V. albo-atrum* isolates to pepper.

Although severe disease expression developed in peppers at soil temperatures of 15–30°C, when the stunting indexes for the 6 varieties are combined and averaged for each temperature, it appears that severe wilt can be expected to develop most consistently between 25° and 30°C. This is a higher maximum for disease development than that reported for tomatoes by Bewley (1), but it is in substantial agreement with Ludbrook's (9) report on wilt of eggplant induced by a microsclerotial strain of *Verticillium* that he identified as *V. dahliae*. Maximum development of Verticillium disease expression in tomato infected with the microsclerotial strain, as reported by Edgington and Walker (3), occurred at soil temperatures similar to those inducing severe pepper disease expression with a comparable air temperature.

Thus, severe Verticillium wilt develops in solanaceous hosts at soil temperatures that correspond very closely to the temperatures at which the pathogen grows reasonably well in culture, e.g. 12–30°C.

The influence of temperature on the relative growth of host and pathogen and subsequent disease development has been clearly elucidated by Leach (8), for several pre-emergence damping-off diseases of spinach, wheat, garden pea, sugar beet, and watermelon. He showed that temperatures favorable for host development but not for pathogen growth, as exemplified by a ratio between host and pathogen growth rates, were temperatures at which pre-emergence damping-off would not develop severely. He was able to state that, in general, when the ratio of growth of host to growth of pathogen was less than 1.0, there was a potentiality for severe pre-emergence infection; as the ratio increased from 1.0 to 4.0, the probability of pre-emergence infection decreased; ratios above 4.0 were associated with total absence of pre-emergence damping-off.

Although it is not necessarily expected that this same relationship might exist in a disease of the wilt-producing type, several observations of Verticillium wilt occurrence make it advisable to examine this

relationship critically. For instance, the early studies in this work showed that plants infected after 60 days' growth were less severely diseased than plants infected after 30 days' growth. Observations in fields planted with tomatoes and peppers in the early spring, when temperatures were less than favorable for their rapid growth, indicate that these plants became more severely diseased with Verticillium wilt than those in the same fields and of the same crops planted in the late spring or early summer, when temperatures were higher and more favorable for host growth.

One would conclude from data shown in Fig. 2 that, in contrast to the results of Leach's (8) study of pre-emergence damping-off, the degree of severity of Verticillium wilt of pepper as expressed by the stunting symptom is related only to the ability of the pathogen to grow, and is not greatly influenced by host growth. If this statement were not true, at least in part, one would expect the most severe expression of Verticillium wilt in these studies to have occurred at a soil temperature of 15°C, with a gradual decline in severity with temperatures increasing to 30°C, and a rapid decline at 35°C. Such was not the case. As shown earlier, the pathogen grows reasonably well at temperatures of 12–30°C, and within this range it appears that degree of disease severity depends more on the relative growth of the Verticillium strain than that of the host.

It will be noted, however, that the host-pathogen ratio curve at 25°, 30°, and 35°C, appears to have a valid inverse relationship to the stunting index curve. In this temperature range the pathogen is progressively inhibited in growth, whereas the pepper host increases in growth rate before it declines at 35°C. Thus, in this range of soil temperatures the application of the ratio to predict disease severity would appear tenable. In actual practice, at soil temperatures above 30°C the Verticillium disease of peppers and tomatoes has been observably less severe, and this host and pathogen temperature relationship seems to account for the success that growers have had in utilizing Verticillium-infested ground for these late-spring- and summer-grown crops.

When the data in Table 3 are examined with respect to varietal differences in disease severity, there does not appear to be any outstanding expression of disease resistance in any of the 6 varieties. Varietal susceptibility therefore seems to be an unlikely explanation of the variation in occurrence of Verticillium wilt of pepper in the areas of commercial pepper production.

The evidence seems conclusive that disease expression results only when peppers are grown in fields where the pepper strain of *Verticillium albo-atrum* exists, and then only when soil temperatures are favorable for pathogen growth. This seems to be a logical explanation of the previously unpredictable occurrence of Verticillium wilt in pepper culture in California. This information makes it possible for the grower to adjust his planting schedule and selection of crops so as to avoid serious losses from this disease.

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A TRANSMISSIBLE DISEASE OF HELMINTHOSPORIUM VICTORIAE[1]

G. D. Lindberg[2]

SUMMARY

Certain abnormally stunted colonies of *Helminthosporium victoriae* were shown to harbor a transmissible agent capable of producing the abnormality in normal colonies. Such normal colonies were induced to grow abnormally upon hyphal contact with abnormal growth and when dipped into a macerated suspension of the abnormal growth. Not all isolates of *H. victoriae* were affected. That bacteria were not the causal agent was concluded from the facts that bacteria were never demonstrated under the microscope or cultivated on artificial media, and streptomycin sulfate failed to destroy the activity of the agent involved. Unlike a toxin, the agent was never demonstrated in media supporting the abnormal growth, and the abnormality was induced in colonies that did not previously show this phenomenon. The activity of a suspension containing the agent was determined by testing against susceptible, normal *H. victoriae*. In such a test, clarified abnormal juice showed slight activity at a dilution of 1:1000, but not at 1:5000. The agent was destroyed by heat at 50 but not at 45°C. The active agent was separated from the fungus by fractionation with ammonium sulfate and centrifugation. An incubation period of at least 24 hours was required before the active agent could be demonstrated in colonies arising from normal fungus dipped into a suspension of abnormal juice. No activity was demonstrated in the filtrates of clarified abnormal juice after passage through bacteriological filters, and the agent has not been identified by electron microscopy.

Among cultures of *Helminthosporium victoriae* Meehan & Murphy, newly isolated from diseased Victor-grain oats, several abnormally stunted colonies were encountered. Closer examination revealed that some of the colonies had started normal growth but that abnormal sectors began to develop, at which time the growth of the entire colony rapidly stopped and the aerial mycelium that had appeared normal collapsed. Subcultures of the 2 colony types—normal and abnormal (stunted)—always yielded colonies like the originals.

An investigation was conducted to determine the nature and cause of the abnormality.

MATERIALS AND METHODS.—A macerated suspension of the abnormal growth, basic in most of the studies, is herein referred to as clarified abnormal juice. The suspension was prepared by scraping abnormal colonies free of agar, macerating with a sterile mortar and pestle, and diluting with sterile water. This material was allowed to settle for 1–2 minutes, and the relatively clear supernatant was drawn off with a sterile pipette.

To determine the activity of the suspension, normal *H. victoriae* was grown on potato-dextrose agar, cut into blocks about 3 mm square, dipped into the suspension, and plated. Data were taken after 72 hours of incubation.

RESULTS.—*Transmission studies.*—Among the isolations of *H. victoriae* in which the abnormality was first detected, there appeared to be transmission of an agent from abnormal colonies to normal colonies, resulting in stunted growth of the latter. Transmission of such an agent was readily tested by placing small blocks of normal and abnormal growth about 1 cm apart in the center of a culture plate of potato-dextrose agar. Abnormal fungus grew very slowly, reaching a diameter of 1–2 cm in several weeks. Normal *H. victoriae* grew very rapidly, though less rapidly in the direction of the abnormal fungus. Within a few hours of contact between hyphae of the 2 colonies, the entire advancing margin of the normal colony stopped growing and the aerial mycelium lysed or collapsed (Fig. 1A). Collapse of the aerial mycelium became complete only when the entire circumference of the colony became affected. If the normal fungus reached the edge of the Petri plate and thus matured before showing signs of abnormality, it remained unaffected, indicating that induction of abnormality was favored by rapidly growing fungus (Fig. 1B). Subcultures of the fungus from these apparently unaffected areas always resulted in normal colonies, whereas transfers from any areas showing abnormality resulted in abnormal colonies.

Normal fungus dipped into a suspension of abnormal fungus resulted in abnormal colonies. This method of transmitting the agent of abnormality provided a simple and rapid technique for assaying suspensions containing the active agent. After incubation for 24 hours, all inoculated colonies still appeared identical to uninoculated controls. The symptoms characterized by abnormal growth were well established after incubation for 72 hours, at which time data from such an analysis were taken.

Tests for the presence of bacteria.—The agent responsible for the peculiar behavior of *H. victoriae* seemed to be most likely explainable on the basis of bacterial contaminants, even though no bacterial growth had been observed in association with abnormality. However, an examination of many Gram-stained and unstained microscope mounts failed to reveal the presence of bacteria. Failures also resulted

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when abnormal colonies were removed aseptically from culture plates, macerated with sterile mortar and pestle, diluted with sterile water, streaked and plated on potato-dextrose and nutrient agars, and incubated at room temperature and 35°C.

One of the most simple and effective methods known for demonstrating bacterial contamination of fungus cultures is by growth of the fungus in broth cultures. But when abnormal fungus was seeded in potato-dextrose and nutrient broth and incubated as described above, all broth cultures remained unclouded, and microscopic examination failed to reveal the presence of bacteria.

Although all attempts to demonstrate bacteria had failed, it was thought that an antibacterial agent such as streptomycin sulfate might indicate the activity of a bacterium by destroying the agent responsible for abnormal growth. Therefore, abnormal fungus was seeded into Erlenmeyer flasks (250 ml) containing 50 ml of potato-dextrose broth or nutrient broth plus 5000 ppm of streptomycin sulfate. Similarly, abnormal fungus was seeded to potato-dextrose agar and to nutrient agar culture plates containing streptomycin sulfate at 5000 ppm. The flasks and plates were incubated at room temperature and 35°C. The resulting cultures, removed after 7 days of incubation, were cut into small pieces, and 20 from each were plated on potato-dextrose agar. Every plating was successful. In no case did the antibiotic affect the activity of the agent when applied to the fungus in culture.

It must be emphasized here that the abnormal cultural characteristics were somewhat accentuated when diseased *H. victoriae* was grown on solid or liquid media in the presence of streptomycin sulfate. When grown in a medium containing the antibiotic, even control cultures of normal *H. victoriae* showed ab-

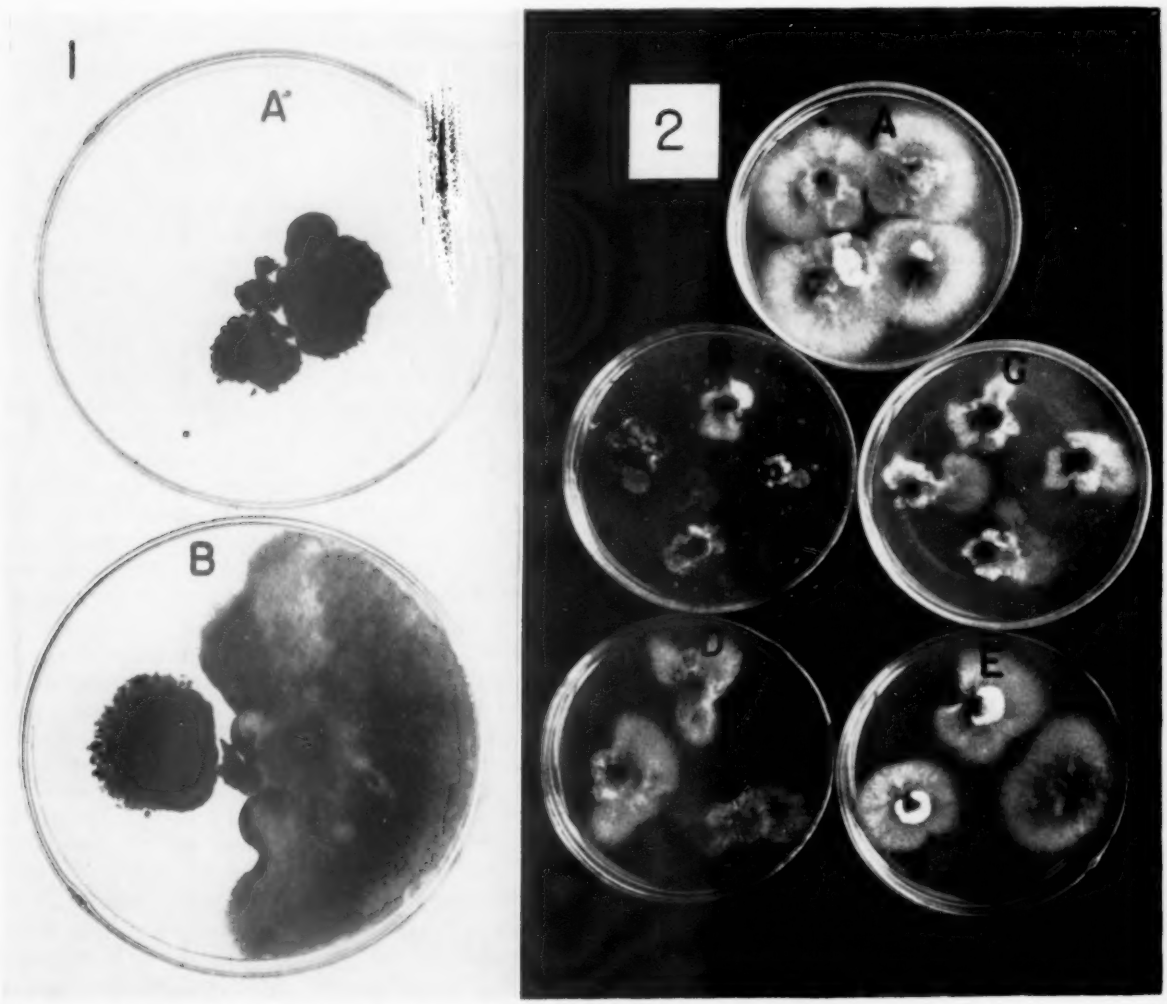

FIG. 1. Normal and abnormal *H. victoriae* seeded in the same plates. A) Entire circumference of the once normal growth (colony at right) is diseased. B) Normal colony has become diseased across the entire advance margin, with lysed aerial mycelium extending back several mm. At far right, normal growth is unaffected. FIG. 2. Dilution series of the agent causing abnormality in *H. victoriae*. A) normal fungus; B) undiluted juice; C) 1:10 dilution; D) 1:100 dilution; E) 1:1000 dilution.

normal cultural characters that may be confused with the transmissible abnormality. However, transfers back to medium lacking the antibiotic always resulted in normal growth.

Tests for the presence of a toxin.—Tests were devised to determine whether the agent causing the abnormal growth of *H. victoriae* had the properties of a toxin. The basic behavior of the abnormality seemed to indicate that the causal agent did not function as a toxin. The fact that the agent could be induced to multiply in isolates (normal *H. victoriae*) that did not previously show this phenomenon appeared to be against the possibility that a toxin was involved.

The media from potato-dextrose and nutrient broth and from potato-dextrose and nutrient agar cultures of abnormal *H. victoriae* were analyzed for activity. In the case of the broth cultures the medium was withdrawn with a sterile pipette, and with solid cultures the medium was taken immediately adjacent to abnormal colonies. These media were tested against normal *H. victoriae*. In no instance was the medium found to have any activity in producing abnormality. This was a strong indication that, if the agent was toxin-like in nature, it was not present in the media supporting the abnormal growth. Further indications were that the causal agent was present only within the fungus or very close to it.

Dilution end point.—Dilutions of the agent causing abnormality in *H. victoriae* were tested against the normal fungus (Fig. 2). Dilutions of 1:10, 1:100, 1:1000, and 1:5000 were made of clarified abnormal juice. All of the colonies arising from normal fungus treated with undiluted juice and the dilution of 1:10 developed diseased sectors, whereas 85% of those at 1:100 dilution resulted in abnormal growth. Only 10% of the test colonies became diseased at the 1:1000 dilution; a dilution of 1:5000 showed no activity.

Inactivation by heat.—Aliquots of clarified abnormal juice were heated 10 minutes at 40, 45, 50, and 55°C to determine the point of inactivation of the causal agent. The agent causing abnormality was highly active at 40 and 45°C, but not at 50 and 55°C, although material heated at 50° developed some abnormal colonies if streaked on culture plates in sufficient quantities.

Filtration.—Clarified abnormal juice was passed through the Seitz filter and a very fine glass filter, and the filtrates were tested for activity against normal fungus. In an attempt to concentrate the active agent, the filtrates in many cases were centrifuged 1 hour at 25,000 rpm (73,300 G) in the Spinco Model L centrifuge. No activity was detected in any of the filtrates, either before or after concentration.

Incubation time for production of the active agent.—Colonies from seeded pieces of normal fungus that had been dipped into abnormal juice were tested for activity at certain intervals after plating. The young colonies were macerated, diluted with a small quantity of sterile water, and tested against normal fungus. The first signs of activity occurred 24 hours after plating, and activity increased noticeably after 30–48 hours. Control colonies were always normal.

Separation of agent from the fungus.—The agent of abnormal growth of *H. victoriae* was separated from the fungus by centrifugation. Centrifuging clarified abnormal juice 5 minutes at 10,000 rpm (11,730 G) sedimented most of the fungus capable of growth. After 0.5 g of ammonium sulfate was added, the supernatant was centrifuged 10 minutes at 15,000 rpm (26,390 G), which apparently sedimented all the fungus although the supernatant retained some of the active agent. High speed centrifugation for 60 minutes at 25,000 rpm (73,800 G) concentrated the agent causing abnormality although the same preparation produced no fungus growth when streaked on culture plates. These results indicate that the active agent was separated from the fungus and that infection of normal fungus by the agent was possible, independent of abnormal growth.

Reaction of normal isolates.—The agent causing abnormality in the isolate of *H. victoriae* presented here does not affect all isolates of the fungus similarly. A study of the agent causing abnormality has not been made with a large number of isolates of *H. victoriae*, but it was found that an isolate from Georgia was unaffected. The Georgia isolate failed to show any abnormal symptoms when treated with the agent of abnormality, and the treated colonies showed no activity when tested against susceptible normal *H. victoriae*.

CONCLUSIONS.—On the basis of the experimental evidence presented above, the agent causing abnormality was considered not to be of bacterial origin or of the nature of a toxin. Bacteria were never demonstrated microscopically or by standard cultural techniques, and streptomycin sulfate did not destroy the activity of the agent causing abnormality. The toxin theory was eliminated, because the active agent could not be demonstrated in any of the media supporting the growth of abnormal fungus and because abnormality was induced in isolates that did not previously show this characteristic.

Luria[3] and Smith and Williams,[4] as well as other virologists, have stated that viruses affecting fungi have not been reported, and that they are either absent, extremely rare, or difficult to detect. That the causal agent is virus-like or an ultra-microscopic organism appears to be the most likely possibility for explaining the nature of the agent causing abnormality in *H. victoriae*.

The reasons against regarding the agent causing the abnormality as a virus are that the agent has not been demonstrated satisfactorily by electron mi-

[3] Luria, S. E. 1953. General virology. John Wiley and Sons, Inc. New York.

[4] Smith, K. M., and R. C. Williams. 1958. Insect viruses and their structure. Endeavour 17: 12-21.

croscopy and that the active agent was not present in the filtrates after passage of the active clarified abnormal juice through the Seitz or the very fine grade bacteriological filters. On the other hand, the evidence in support of regarding the agent as a virus or as an ultra-microscopic organism are that the agent 1) did not yield to cultivation on artificial media, 2) was not demonstrated in Gram-stained or unstained mounts with the light microscope, 3) was for the most part destroyed by heat at 50°C., and 4) showed slight activity at a dilution of 1:1000 from clarified abnormal juice. In addition, it has been shown that an incubation period of 24 hours occurred before activity could be demonstrated in those colonies arising from normal fungus dipped into abnormal juice. This is strong evidence that the infective agent requires a developmental and maturation period closely simulating a life cycle.

Only a comprehensive survey could reveal the extent to which other fungi may be affected by similar transmissible abnormalities. Some of the sectoring in the fungi that is so commonly attributed to mutation or other chromosomal aberrations may be due to transmissible abnormalities similar to that described in this manuscript.

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A PHYSIOLOGICAL BASIS FOR RESISTANCE OF OATS TO VICTORIA BLIGHT[1]

R. R. Romanko[2]

SUMMARY

Victorin, the toxin produced by *Helminthosporium victoriae*, was used in a laboratory study of disease resistance. Seedling bioassays were used as quantitative and qualitative tests for victorin recovered in plant-extract–toxin mixtures obtained by 1) making extracts from cuttings that had taken up victorin, and 2) adding victorin to prepared plant extracts. Following uptake by intact plant tissues, victorin could be recovered regularly only from extracts from plants of varieties susceptible to Victoria blight; when mixed with plant extracts, victorin was recovered regardless of extract source. The data indicated that victorin was inactivated by intact oat tissue of varieties resistant to Victoria blight. Such inactivation of victorin, produced *in vivo*, would account for disease resistance in the field. Following treatment with victorin, susceptible oats showed marked increases in the respiration of leaves, roots, and entire seedlings; resistant oats did not. This is apparently the first report of a toxin producing such an effect only in plants susceptible to the pathogen.

Previous studies of Victoria blight of oats have provided reasonably clear evidence that a valid study of the physiology of this disease could be made by using the toxin produced *in vitro* by the causal organism, *Helminthosporium victoriae* Meehan & Murphy.

Meehan and Murphy, who first suggested that a toxin was involved, reproduced disease symptoms using an *in vitro* toxin and showed that varietal reactions to both the fungus and its toxin were identical (6, 7, 8). Their findings were confirmed by Luke and Wheeler, who also correlated isolate pathogenicity and growth with *in vitro* toxin activity (5). These workers concentrated the active principle (termed victorin) by a process involving elution of the adsorbed victorin from norite with an HCl-acetone mixture. In the published abstract of their results (10) these authors omitted mention of the HCl in the eluting solution. This undoubtedly accounted for Pringle and Braun's failure to obtain similar concentration and purification by this process (9). The latter devised another method by which they were able to isolate victorin, and obtained polypeptide material with high specific activity.

Both Luke and Wheeler and Pringle and Braun developed seedling bioassays that were sensitive to minute quantities of active victorin. Through their assays the former group was able to detect victorin in 2-day-old culture filtrates of *H. victoriae*, thus seemingly eliminating the possibility that the toxin produced was a lysis product of aged cultures. They also obtained culture filtrates that were inhibitory to the roots of susceptible seedlings at dilutions 10^6 times that which would inhibit roots of resistant plants. Moreover, after purification, it appeared that victorin itself was innocuous to resistant plants and that inhibition by the crude filtrate was due solely to a secondary nonspecific toxin of low activity, such as has been described for *H. sativum* Pam, King, & Bakke (4). Extending the study of victorin to other cultivated plants, these workers found that its activity was restricted to oats, and solely to varieties possessing the Victoria hypersensitive crown-rust reaction. However, selections from these varieties were later obtained that were unaffected by victorin and possessed resistance to both *H. victoriae* and the crown rust organism, *Puccinia coronata* Cda. (2, 11).

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This indicated that the inheritance of disease resistance was more complex than the single pleiotropic gene postulated by Litzenberger (3).

To summarize: An impressive array of facts indicates that victorin is primarily, if not solely, responsible for the symptoms of Victoria blight of oats. Both victorin and *H. victoriae* produce similar disease symptoms, both show the same remarkable pathogenic specificity, and victorin is produced most rapidly by actively growing cultures and in direct proportion to the pathogenicity of the isolate involved. Crude victorin possesses very high biological activity, and purification increases both its activity and specificity. Moreover, there is some evidence that victorin can be recovered from diseased tissues not occupied by the fungus. This evidence is circumstantial rather than direct and conclusive, but it seems sufficiently extensive to justify the use of victorin in studies of the physiology of this disease. One obvious approach involves investigation of the nature of resistance and susceptibility to this toxin.

In view of the remarkable biological activity and unique specificity of victorin, it seemed likely that the wide range of plant species and non-Victoria oat varieties resistant (or tolerant) to victorin possess this quality as a passive attribute, and that victorin is not a toxin *per se*, but in some manner contributes to active formation of a toxin, possibly a reaction product formed only *in situ*, in plants possessing the susceptible Victoria genotype. The reaction in such an event would be active susceptibility, with passive resistance residing in all plants, *Avena sativa* or others, that lack the physiological mechanism inherent with the Victoria genotype. The study herein reported was an inquiry into the basis of the action of victorin, and into the properties of oats that might account for resistance or susceptibility, whether active or passive.

MATERIALS AND METHODS.—The seedling bioassay method developed by Luke and Wheeler (5) was used as a test for victorin recovered from cuttings previously exposed to victorin solutions or for victorin present in victorin–plant-extract mixtures. Root inhibition specific to susceptible seedlings served as a qualitative test for victorin. The degree of inhibition, over and above that exerted by control extracts or mixtures, was used for quantitative estimates of the amount of victorin present. Cuttings were made from resistant (Camellia) and susceptible (Victorgrain 48-93, Park) oat varieties and placed in victorin dilutions for designated periods (1–24 hours). After exposure, the basal portion of the cuttings was removed; and the upper portion, which had not been in physical contact with the solutions, was macerated and extracted with distilled water (sometimes weakly acidified with HCl or buffered solution (McIlvaine's standard buffer, made to pH 5.6 and diluted 1:29 with distilled water). The extracts thus obtained were bioassayed for the presence of victorin. In another group of experiments, measured quantities of victorin were added to previously macerated oat materials, and the mixtures were bioassayed after an incubation period.

In the third group of experiments, the effect of victorin on rate of aerobic respiration was determined in a Warburg apparatus. Oats were exposed to victorin either as cuttings or as seedlings. In some of these tests vacuum infiltration was employed to establish simultaneous contacts of all tissue with victorin.

EXPERIMENTAL RESULTS.—Bioassays of extracts obtained from cuttings exposed to victorin showed that a specific toxin was regularly recovered from susceptible cuttings, but not from resistant cuttings. All control extracts showed a low degree of nonspecific toxicity. Specific toxicity, over and above the control, was rarely present in the extract of exposed resistant cuttings, and when it did occur it represented less than 10% of the estimated recovery from susceptible cuttings. The specific toxicity recovered in these tests was assumed to be victorin, since only susceptible seedlings were inhibited.

Fig. 1A shows the root growth of resistant and susceptible seedlings grown in extracts obtained from susceptible cuttings that had been allowed to stand in victorin solutions 1, 2, 4, 8, 16, and 24 hours. These results illustrate that the growth of resistant seedlings was at least as great in the extracts from exposed cuttings as in the controls, and the growth of susceptible seedlings was progressively inhibited as the dilutions decreased. Although the range of inhibition at a given dilution was rather wide in some cases, there was no evidence that variation in exposure period was responsible. The facts may or may not be significant that resistant seedlings grew faster than the controls at all dilutions tested, and susceptible seedlings only at the highest dilution. Similar results in other experiments suggest that victorin may have stimulatory effects on resistant plants (and possibly on susceptible ones at very high dilutions). However, such effects have always been minor, and not consistently reproducible.

From the results obtained, it is apparent that a specific inhibitory agent was present in the extract of susceptible cuttings exposed to victorin, and that this agent possessed the same specificity as victorin. It is also apparent that there was no significant accumulation of this toxic substance, indicating that inactivation occurs to a degree comparable to that which ordinarily occurs when victorin stands at room temperatures in less than moderately acid solutions (i.e., at a pH above 4.0). Such a condition obviously occurs in plant sap.

Since the resistant Camellia seedlings showed no inhibition other than that exerted by the control extracts, there was no evidence to indicate the presence of a nonspecific toxic reaction product, the effects of which would have been masked in susceptible seedlings by a mere trace of victorin in the extract.

Fig. 1B typifies the results obtained when both resistant and susceptible cuttings were exposed to a

victorin solution. In this test only susceptible seedlings were used to bioassay the extracts. Camellia and Victorgrain 48-93 cuttings were used as resistant and susceptible sources of extracts. As in all tests, the control extracts were obtained from cuttings that took up water rather than a solution of victorin. As is shown, the extract from exposed Camellia cuttings was no more toxic than the control extracts, whereas the Victorgrain 48-93 extracts were highly inhibitory to assay seedlings at the higher concentrations. In this particular test, the cuttings were allowed to take up victorin solution for 4 hours. The ratio of ml solution taken up per g of plant tissue was 0.14:1 for the Camellia cuttings, and 0.12:1 for the Victorgrain cuttings. In terms of toxic activity and uptake, the Camellia cuttings took up a minimum of 7.5 units per g, with no recovery detectable, whereas the Victorgrain cuttings took up 6.0 units per g, with a recovery estimated between 1.6 and 7.9% (5). The recovery of victorin was quite varied. In different tests the detectable recovery ranged from less than 1% up to 42%. Though this recovery may seem inefficient, the demonstrated instability of victorin must be considered, and its adsorptive properties as well (5, 9). It was found that efficiency of recovery was maximum when exposure was relatively short, when exposed cuttings were promptly frozen, and when extraction was conducted at low temperatures with an acidified solution.

Bioassays of residual solutions (solutions of victorin to which cuttings had been exposed) showed that exposures of 24 hours reduced toxic activity 90–99%. Quantitatively, the toxic activity lost was the same in the residual solutions from cuttings of both resistant and susceptible varieties. Hence, selective uptake of victorin by susceptible plants, or selective rejection, or inactivation by discharged fluids, by resistant plants did not appear to be a factor in recovery of the toxin in one case and lack of recovery in the other.

In view of the virtually complete disappearance of victorin taken up by resistant Camellia cuttings and the consistent recovery from susceptible Park and Victorgrain 48-93 cuttings, tests were designed to establish whether mechanical mixtures of plant extracts and victorin would yield similar results.

Victorin dilutions in a wide range were added to previously obtained extracts of plant tissue. The conditions of these tests were varied by conducting them at different temperatures, by providing varied incubation periods before bioassaying, and by utilizing whole plants, or roots, or shoots, and plants and plant parts of different ages; in addition, the amount of active victorin in the higher dilutions used was much less than the amounts taken up and inactivated by Camellia cuttings; yet there was no indication of any inactivation in these mixtures.

In certain of these tests, a 24-hour incubation of victorin–plant-extract mixtures before bioassay produced additive or synergistic toxic effects, resulting in apparent hundredfold increases in victorin activity. These results were, however, inconsistent, and the conditions and mechanisms involved were not clarified.

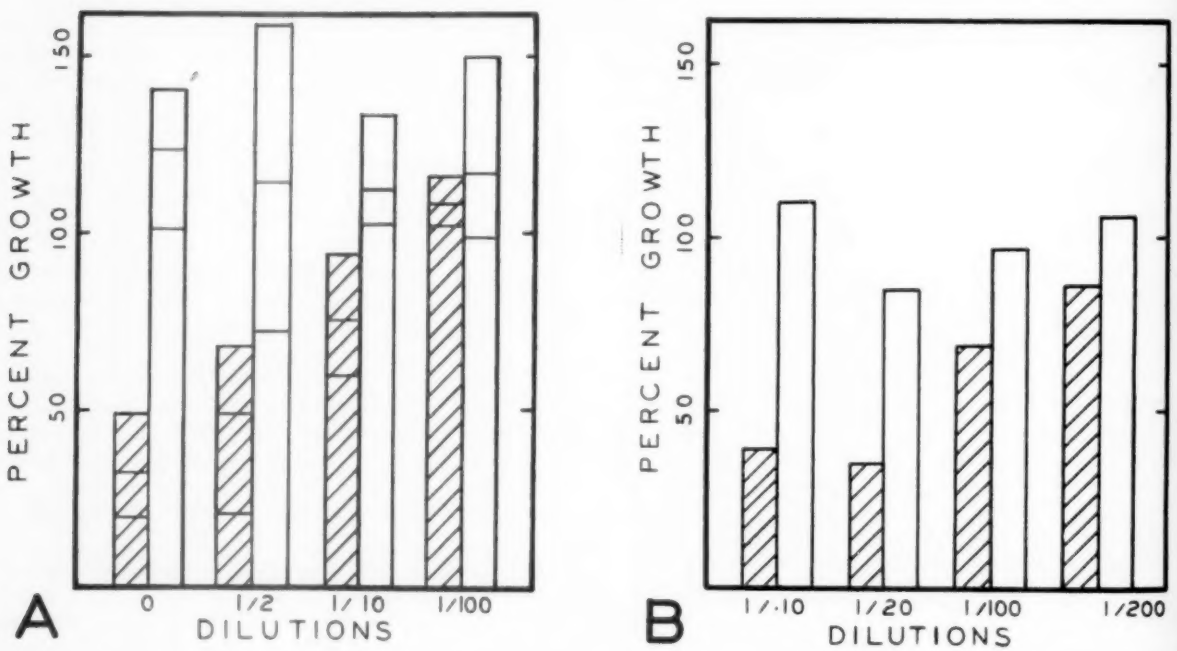

FIG. 1. Root growth as per cent of control. A) Root growth of seedlings resistant (white bars) and susceptible (striped bars) to Victoria blight grown in extracts obtained from susceptible cuttings exposed to victorin (the active toxic principle). Variation of growth in extracts differing by the length of exposure to victorin is shown by horizontal crossbars, delimiting range and showing mean. B) Root growth of seedlings susceptible to Victoria blight in extracts obtained from resistant (white bars) and susceptible (striped bars) cuttings exposed to victorin for 4 hours.

TABLE 1.—*Respiration ratings of Victorgrain 48-93 and Camellia cuttings after exposure to victorin*

Exposed cutting	Control (= 100)	Hours cuttings exposed			
		2	4	10	24
Victorgrain	Victorgrain (not exposed)	149	209	225	35
Camellia	Camellia (not exposed)	113	100	91	90
Victorgrain	Camellia (exposed)	144	214	235	31

Since complete inactivation of victorin proceeded only in intact cells of the resistant variety, and since this indicated possible differences in the metabolism of resistant and susceptible varieties, a third group of experiments was concerned with the effect of victorin on respiration. Table 1 summarizes the results of 12 tests in which cuttings of Camellia and Victorgrain 48-93 were exposed to 1:10 dilutions of victorin. The victorin solution used in these tests had given 50% inhibition of root growth of susceptible seedlings at a dilution of 1:30,000. Dilute buffer solution or heat-inactivated victorin diluted 1:10 served as controls. After exposures of 2–24 hours, uniform sections were removed from several cuttings, 8–10 in. from the submerged basal end, and the respiration was measured. All determinations were made at 25°C and the data converted to mm^3 gas per g dry wt. As may be noted, the respiration of exposed Camellia leaf tissue was essentially unaffected by uptake of victorin, whereas the respiration rate of Victorgrain 48-93 leaves was considerably increased, becoming 2–3 times those of both Victorgrain controls and of treated and untreated Camellia. The respiration quotient in these tests was a little below 1.0 and usually decreased as the exposure periods lengthened, but was essentially the same for all treatments.

Respiration was also measured in roots, shoots, and whole seedlings, both while suspended in victorin solutions and after vacuum-infiltration of tissues. The same increase in respiration resulted. Response to victorin was more rapid in the roots of susceptible plants than in green tissue, but after maximum respiration was reached (usually 4–10 hours after exposure to victorin), respiration declined in all materials, leveling off at 30–40% of that of controls.

In none of these tests was sufficient material added (e.g., buffering solution) to produce any effects from its utilization as a substrate; and since there was always a small decrease in fresh weight and no measurable elongation, growth cannot be considered a factor in the increased respiration, which in a few instances surpassed the control rate by 4 or 5 times.

Fulgrain, another variety susceptible to Victoria blight, showed the same respiratory response as Victorgrain 48-93.

Because the known respiratory uncoupler, DNP (2,4-dinitrophenol), incites marked increases in respiration when applied in low concentration, it was used in a comparison with victorin on susceptible cuttings.

Cuttings were left in victorin and DNP (10^{-4} and $10^{-5}M$) for 4 hours in bright sunlight, and sections of leaf were then removed. Rate of respiration was measured both immediately after exposure and after an interval of 6 hours. All cuttings wilted similarly, but the respiratory response elicited by DNP was mild in comparison with that caused by victorin (Table 2).

A determination on Victorgrain 48-93 oat leaves that expressed symptoms from natural infection by *H. victoriae* indicated that respiration increased in diseased plants also.

DISCUSSION.—Bioassays of extracts from victorin-exposed cuttings indicated that active resistance was the governing factor in the reaction of oat varieties to victorin. The selective toxicity to susceptible seedlings manifested by extracts obtained from exposed susceptible cuttings showed that victorin was recovered from these cuttings. Inasmuch as measurements of uptake and bioassays of residual victorin solutions showed no evidence of quantitative differences in uptake or of differences in the victorin activity of the residual solutions, the failure to recover victorin from resistant Camellia cuttings appears due to inactivation of victorin by these cuttings. Since there was no indication of a third nonspecific toxic principle in the extracts of exposed susceptible cuttings, there appeared to be no foundation for the premise that the specific action of victorin involved participation in the formation of a toxic product by the susceptible host. The failure of plant material extracts from resistant cuttings to inactivate victorin, in contrast to the ready inactivation that occurred in cuttings prior to extraction, indicates that inactivation may not result from a simple one-step reaction but probably as a result of interactions that can occur only in intact cells.

The heightened respiration of susceptible plant tissue, coupled with the eventual drop to 30% of initial or control respiration, seemed to indicate one of two empirical conditions:

First, it is possible that only about 70% of the cells from susceptible plants were affected by victorin and that the respiration rate of these cells was substantially greater than the data of the entire tissue would indicate. In this event, the decline of respiration would indicate a cessation through the death of

TABLE 2.—*Respiration rates of Victorgrain 48-93 tissue after 4 hours of exposure to victorin and DNP (2,4-dinitrophenol)*

Treatments	Hours after end of exposure			
	O_2 consumption[a]		% of control	
	0	6	0	6
Victorin (partially purified)	242	213	425	533
DNP ($10^{-4}M$)	101	65	177	162
DNP ($10^{-5}M$)	82	52	144	130
Buffer solution (control)	57	40	100	100

[a] In mm^3 per hour per g dry weight.

affected cells, and the continued low rate would represent the unaffected respiration of the cells that were insensitive to victorin or, for reasons possibly related to differences in permeability, had escaped its effects. This seems unlikely in view of the results with seedlings and roots where long exposures did not diminish the rate below 30% although infiltration of all cells with victorin probably occurred. However, as Gaumann recently indicated, pH may vary in different cells, and this variation might well govern the permeability of cells to toxins (1). Since pH is a demonstrably critical factor in the stability of victorin, this first condition cannot be considered invalid.

The second general condition would involve the presence of alternate metabolic pathways such as those demonstrated to be operative in the formation of pentoses. In this eventuality, all cells would sooner or later be affected by victorin, and the increase, drop, and leveling off of respiration would in such case represent the stimulation, and eventual complete inactivation, of only that portion of the metabolic system of every cell upon which victorin has an effect. Sustained respiration at 30% of that of controls would in such an event represent the functioning of an alternate system unaffected by victorin, a functioning that would, however, be inadequate, only delaying the ultimate death of the entire tissue.

Although the increased respiration by susceptible tissues suggests the uncoupling of phosphorylation from oxidation, the smaller increase effected by DNP shows that the mode of action is probably not the same. It is likely, however, that even low concentrations of DNP will exert an effect at more than one metabolic site, so that resultant respiratory increases probably represent a net effect. On the other hand, the specificity of victorin and the reported monogenic inheritance of host reaction suggest that its activity may well be confined to a single metabolic site or mechanism.

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SEXUALITY OF PHYTOPHTHORA PARASITICA VAR. NICOTIANAE[1]

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SUMMARY

More than 200 isolates of *Phytophthora parasitica* var. *nicotianae* were checked for oospore production in single culture and in matings with tester lines. Thirty isolates produced oospores when grown in single cultures. Some isolates of the flue-cured and burley strains derived from single zoospores produced oospores promptly and abundantly at 20°C on oatmeal agar, whereas others produced them erratically. Most isolates failed to produce oospores in pure culture, but were compatible when appropriately mated. Isolates of the flue-cured strain of the black shank fungus were cross compatible with most isolates of the burley strain and with an isolate of *Phytophthora capsici* from squash. Isolates of the burley strain were not cross-compatible with *P. capsici*. It is probable that isolates of the flue-cured strain function as the antheridial parent when paired with *P. capsici* or with isolates of the burley strain. Consequently, most isolates of the latter type are oogonial. Oospores were observed to germinate on rare occasions, but no isolates from single oospores were established, thus precluding genetic analysis of the sexual mechanism and making it impossible to state specifically whether the black shank fungus is homothallic or heterothallic. Microscopic observations of developing oogonia support the interpretation that the oogonial initial penetrates the antheridial initial during oogenesis.

Black shank, caused by *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker, is potentially the most destructive disease of tobacco in the southeastern United States. Tobacco culture in North Carolina today largely depends on disease-resistant varieties (11). Consequently, any pathogenic variability of the causal agent should be considered to help ensure continued success of this disease control program.

Natural populations of *P. parasitica* var. *nicotianae* exhibit extreme variations in cultural morphology and pathogenicity (2). In culture, isolates from the burley-tobacco-producing areas of Kentucky, Tennessee, and western North Carolina differ significantly and consistently from isolates collected in the flue-cured-tobacco areas of North Carolina and adjoining states. These 2 types have been designated as distinct cultural strains: the burley strain and the flue-cured tobacco strain. Isolates of the black shank fungus from natural populations also may vary widely in pathogenicity. At least part of this variability is due to mutation (2); however, little is known about the oosporic stage as a source of variation through recombination.

Oospores of the black shank fungus have been found infrequently in culture, and not in nature. Ashby (3), Tucker (14), and Kouyeas (8) observed sparse to moderate oospore production in culture. Several investigators (8, 9, 13) obtained oospores in matings of *P. parasitica* var. *nicotianae* with *P. parasitica*. Johnson and Valleau (7) demonstrated cross-compatibility between isolates of the black shank fungus from Kentucky, but found no self-fertile strains. Kouyeas (8) demonstrated cross-compatibility between isolates of *P. parasitica* var. *nicotianae* from Greece.

Since no comprehensive report of the sexuality of the black shank fungus is available, the present study was initiated to determine if it is homothallic and/or heterothallic, and to assess the relative importance of the perfect stage as a potential source of variability. A preliminary report has been presented (1).

MATERIALS AND METHODS.—The isolates of *P. parasitica* var. *nicotianae* used in this study were obtained as follows: 184 from 55 counties in North Carolina, 1 from California, 10 from Florida, 4 from Georgia, 8 from Kentucky, 6 from Tennessee, 4 from Virginia, 1 from Java, and 1 from Puerto Rico. The isolates of *Phytophthora capsici* Leon. were obtained from pepper (№235) and squash (№209) in North Carolina.

Isolates were paired or grown singly on oatmeal agar at 20°C for a minimum of 2 weeks before being checked for oospore production. The relative degree of sporulation[2] was determined by using a ruled eyepiece reticule to make oospore counts. Bits of mycelium were removed from the paired cultures in the zone of contact and thoroughly squashed with a flat needle. A coverslip was added and pressed against a blotter to flatten the preparation. The average number of oospores in an area of 0.042 mm² on a slide was recorded, and the culture was rated on the following scale: 0 = no oospores; I = <1; II = 1–6; III = 7–14; IV = 15–22; V = 23–30; VI = >30.

EXPERIMENTAL RESULTS.—*Matings among nonsporulating isolates.*—No oospores developed in pure cultures of the first isolates collected. This suggested the existence of self-sterile strains and prompted mating studies, the first of which involved all possible paired combinations of 11 isolates including 10 isolates of the flue-cured strain and an isolate of *P. capsici* (209) from squash. Oospores were formed

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[2] Throughout this paper, "sporulation" refers only to production of the perfect stage.

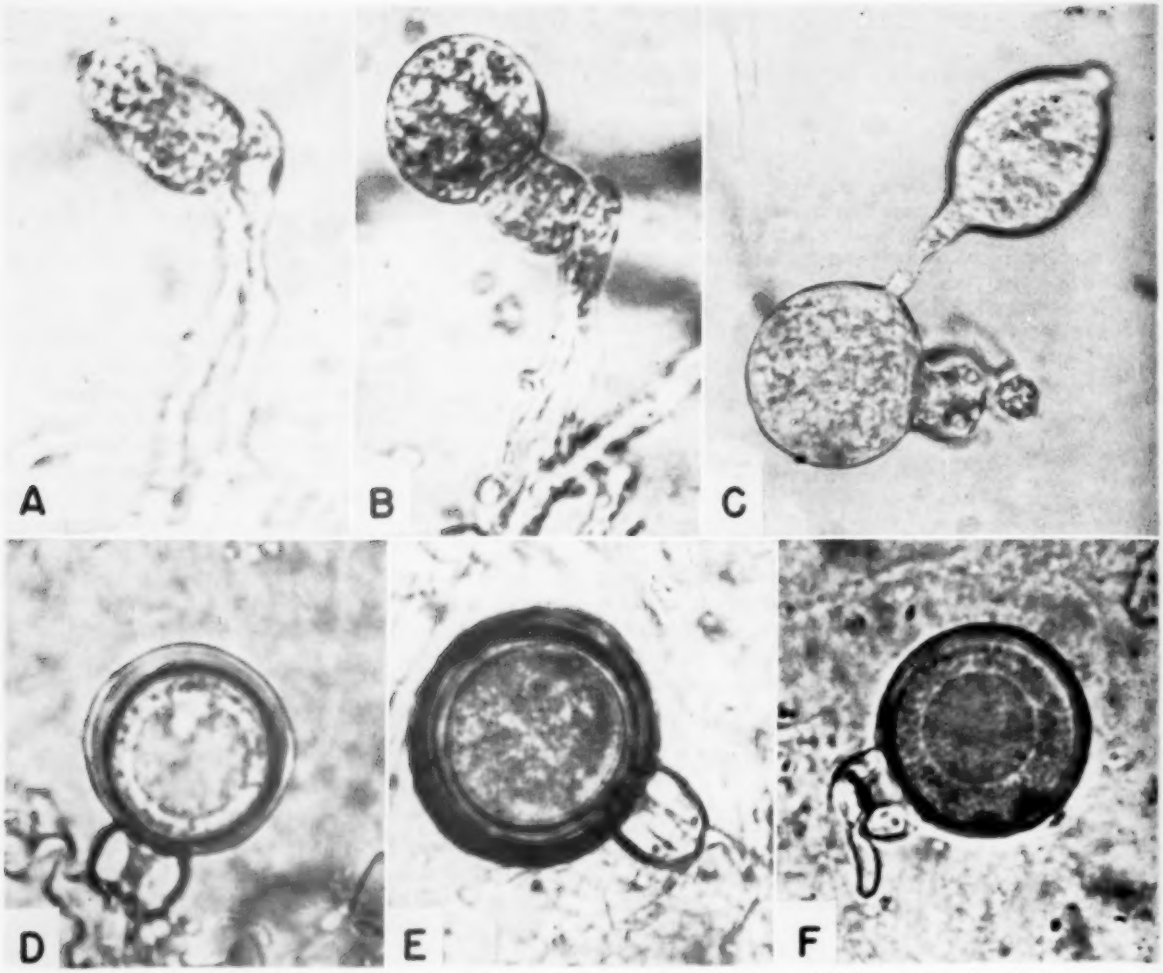

FIG. 1. A, B) Oogonial development in mated cultures of 2 *P. capsici* isolates (209 × 235). A) Photomicrograph taken about 1 hour after the gametangia met. The oogonial initial has already grown through the antheridium and has begun to form the oosphere. B) Photomicrograph of the same developing oosphere taken 2½ hours later (×800). C) Germinated oospore from a mated culture of *P. capsici* (209) × *P. parasitica* var. *nicotianae* (906) (×850). D) Oospore of homothallic isolate of the flue-cured strain of the black shank fungus. E) oospore of the type produced infrequently in pure culture by *P. capsici* (209). F) Oospore of cross of *P. capsici* (209) × *P. parasitica* var. *nicotianae*.

only in combinations involving isolate 209.

Similar mating tests with other isolates of the flue-cured strain produced the same results: oospores were formed only in combinations involving 209, often so abundantly that mating zones were visible at the point of contact (Fig. 2B), and none were formed when 2 black shank isolates were paired. However, when an isolate of the burley strain of the black shank fungus (210) was included in a test, the results differed. Oospores were produced in combination with isolates of the flue-cured strain but failed to occur when 210 was mated with 209. Additional isolates from the burley tobacco area behaved similarly in mated cultures.

Since all nonsporulating isolates of the flue-cured strain produced oospores when paired either with the isolate from squash or with isolates of the burley strain, all new accessions, if not homothallic, were mated with isolates 209 and 906, the latter a black shank isolate of the flue-cured strain highly compatible with 209. Different crosses varied widely in the number of oospores produced. Considering the number of spores produced in paired cultures as a measure of the degree of compatibility,[3] oospore counts were recorded on a scale of 0-VI, from none to very abundant. Using test isolates 209 and 906, 180 nonsporulating isolates of the flue-cured strain were screened for mating type. According to their degree of compatibility, the 180 isolates were grouped into classes as follows: 2 isolates in class 0, 6 in I, 60 in II, 67 in III, 24 in IV, 18 in V, and 3 in VI. No oospores were formed in the unpaired checks.

From these paired culture studies, 2 distinct compatibility groups were evident among the black shank

[3] "Compatibility" is used both in a quantitative sense, denoting degree of sporulation, e.g., weakly compatible and highly compatible; and in a qualitative sense, denoting fertile combinations.

isolates. One group, comprising those mating with 209 but not with 906, was composed of isolates of the flue-cured strain from California, South Carolina, Pennsylvania, Maryland, Virginia, Java, Sumatra, Puerto Rico, Florida, Georgia, and North Carolina. A second group, comprising those mating with 906 but not with 209, was composed of isolates of the burley strain from Tennessee, Kentucky, North Carolina, Florida, and Georgia.

Thus, with respect to mating type, the burley strain appeared opposite to but compatible with isolates of the flue-cured strain. To test this assumption further, 13 nonsporulating isolates of the burley strain were used in more extensive mating studies with flue-cured isolates (Table 1). No isolate of the burley strain formed oospores in combination with all flue-cured isolates, and only 1 of the latter (303) was compatible in all interstrain combinations. Considering all matings, 352 (from Tennessee) was most compatible with flue-cured isolates. In subsequent tests, 352 produced a few oospores in single culture, although none were present in the unpaired plates accompanying this experiment. However, the number of oospores produced was much greater when it was paired with other strains than when it was grown alone. The compatibility of all matings of burley × flue-cured was low in comparison with the general compatibility level between 209 and flue-cured isolates.

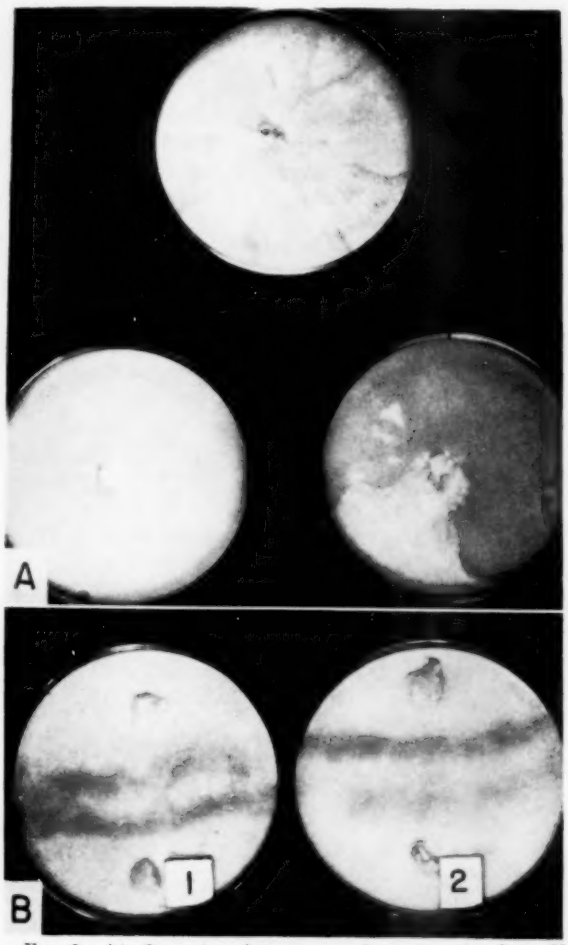

FIG. 2. A) Sectoring for oospore formation in the self-fertile mass-transfer isolate 375. Top plate: mass-transfer culture 375, with areas of depressed mycelial growth in which oospores are abundant. Bottom, right: subculture from the sporulating area that continued to sector for sporulation. Bottom, left: subculture from the nonsporulating area that showed homogeneous vegetative growth and produced no oospores. B) mating lines due to abundant oospore production in the zone of contact between *P. capsici* (209) and *P. parasitica* var. *nicotianae* (906).

Oospores produced in mated cultures differed in size and appearance. Spores obtained from matings involving 209 *(P. capsici)* had hyaline to slightly tan oogonial walls, with the oospore almost completely filling the oogonial cavity. These oospores had relatively thick (1–2 μ), slightly tan but transparent walls (Fig. 1F) and averaged about 30 μ in diameter (Table 2). Oospores formed in matings of burley × flue-cured isolates averaged about 24 μ in diameter, with oogonial walls that were hyaline or brown, both types sometimes occurring in the same plate.

It was not possible to determine definitely whether oospore formation in matings of isolates of the flue-cured strain and *P. capsici* was due to hybridization between these 2 species or to chemical stimulation of one thallus by another. Efforts to trace the gametangial initials to their origins were unsuccessful. Numerous and persistent attempts were made to germinate these oospores, since segregation data from cultures of single-oospore origin should readily reveal whether hybridization had taken place. Oospores from a 2-week-old culture of the 209 × 906 cross germinated in considerable abundance in distilled water on one occasion, but attempts to establish cultures from them were unsuccessful. Some of these germinating oospores formed a sporangium on a short sporangiophore (Fig. 1C), whereas others formed a long germ tube, which did not terminate with a sporangium. Some oospores germinated in both ways.

The possibility that chemical stimulation might play a role in oospore production was investigated by mating and growing separately isolates 209, 235 (an isolate of *P. capsici* highly compatible with 209), and 906 in water cultures on sterilized hemp seeds. After 2 weeks, oospores had formed only in the mated cultures. The liquid portion was passed through a Seitz bacteriological filter to free it of mycelium and/or spores. The resulting filtrates were used to establish new cultures of the parental isolates in all possible combinations of filtrate × parental isolate. No oospores were present after 3 weeks.

Oospores were produced when isolates of the flue-cured strain were paired with isolates of *P. capsici* from Florida (№261) and New Mexico (№264A). These oospores were comparable in size and appearance to those produced in matings with 209 (Table 2). Neither 261 nor 264A produced oospores in single culture.

It was realized that other possible mating types among flue-cured strains might not be detected by using only 2 test isolates. This was investigated in a limited way by pairing 12 isolates of the flue-cured strain in all possible paired combinations. Six of these isolates, previously regarded as self-sterile, produced oospores in single culture. Matings among the 6 nonsporulating isolates (Table 3) indicated other mating types. Isolate 318 was weakly compatible with 4 of the other 5 isolates.

A second experiment of this nature was conducted with 11 isolates from widely separated geographic areas. Three of these isolates were self-fertile, although previously determined as nonsporulating. These results (Table 3) also indicate the existence of other mating types. Isolate 416 (from Puerto Rico) mated with 209, not with 906, and in this experiment mated with all isolates.

Self-fertile isolates of the black shank fungus.—Thirty isolates produced oospores in single culture. Of this number, 6 were of the burley strain but they were sporadic in producing oospores. Small numbers were found in old plates or tubes. Only 1 isolate produced them in considerable quantity (class II); however, it failed to produce them consistently. By contrast, many of the self-fertile isolates of the flue-cured strain produced oospores consistently and abundantly. Several of these were self-fertile when isolated; however, as previously noted, many exhibited self-sterility when isolated but, months later, would produce oospores abundantly in 4-week-old cultures.

The oogonia produced by self-fertile flue-cured isolates were hyaline, with transparent, slightly tan to hyaline oospores (Fig. 1D) of mean diameter about 24 μ (Table 2). The oospores did not fill the oogonial cavity. The oospores produced by burley isolates were similar in size but frequently had brown-pigmented oogonial walls.

Several self-fertile isolates of the flue-cured strain sectored for oospore production (Fig. 2A). The tan sporulating sectors with an abundance of oospores and no aerial mycelium contrasted sharply with the nonsporulating sectors with profuse, aerial mycelium. Transfers from the sporulating sectors gave rise to sectoring colonies, whereas transfers from the nonsporulating sectors gave rise to homogeneous nonsporulating cultures.

A mass-transfer isolate of the flue-cured strain (375) that consistently exhibited this sectoring phenomenon was studied in considerable detail. A series of rapid successive transfers obtained both homogeneous nonsporulating and nearly homogeneous sporulating cultures. From each type, single-zoospore isolates were obtained, 18 from the nonsporulating (designated as 375-NS) and 17 from the sporulating type (designated 375-S). These were plated out on oatmeal agar, but all were apparently self-sterile, since no oospores were produced in 30 days. This prompted mating studies among the 375-S group in an effort to find compatible mating types. They were also mated with the 2 test isolates, 906 and 209. At this time, 2 of these single-zoospore isolates were self-fertile. The 375-NS group was checked in a similar manner; however, all but 3 of this group exhibited self-fertility. Of the 3 remaining nonsporulating isolates, 375-NS1 was compatible only with 209, and 375-NS9 and 375-NS14 were compatible with both 906 and 209. Contrary to expectation, none of these single-zoospore isolates mated only with isolates of the 906 mating type.

Ontogeny of the sexual organs.—Persistent efforts were made to observe the initial copulation of male and female gametangia in mated cultures. The objective was 2-fold: 1) to verify or refute the theory advanced by Pethybridge (12) and Dastur (5) that the oogonial incept actually penetrates the antheridial initial during oogenesis, giving rise to the amphigynous antheridium; and 2) to trace the gametangia to

TABLE 1.—*Oospore production in paired cultures between isolates of the flue-cured and burley strains of Phytophthora parasitica var. nicotianae*[a]

Burley strain isolates	Isolates of the flue-cured strain								
	209[b]	906-9	906-12	303	341	363	369	396A	330[c]
210	0	I	0	II	0	I	0	0	0
216	0	I	0	II	I	II	0	0	I
220[d]	0	III		II		0		0	
333	0	0	I	II	0	0	II	II	I
334	0	I	II	III	0	I	I	I	II
337	0	0	I	II		0	0	0	0
339	0	I	0	II	0	0	0	0	I
340	0	I	0	II	0	0	II	0	I
345	0	I	I	II	0	0	I	0	0
352	0	V	V	II	III	II	III	I	0
353	0	I	I	II	0	0	I	0	0
354	0	0	0	I	0	0	0	0	I
379	0	I	0		I	0	I	II	

[a] Number of oospores in an area of 0.042 mm^2 in the mating zone: 0 = none; I = <1; II = 1-6; III = 7-14; IV = 15-22; V = 23-30; VI = >30.
[b] Isolate of *P. capsici* that mates with flue-cured isolates.
[c] A burley strain secured from E. M. Johnson and designated as antheridial.
[d] Isolated from soil obtained from the cigar-wrapper area of Florida.

TABLE 2.—*Mean diameters*[a] *of oospores from self-fertile and paired cultures of Phytophthora parasitica var. nicotianae*

	Cross or pure culture	Oospore mean (μ)
Matings between P. capsici and isolates of the flue-cured strain	209 × 303	30.7
	209 × 341	31.6
	209 × 906-9	30.6
	209 × 375-S10	31.3
	261 × 411	30.1
	264A × 906	31.4
Matings between isolates of the burley and flue-cured strains	220 × 303	24.7
	334 × 303	24.6
	353 × 303	24.6
	352 × 341	21.2
	352 × 906-9	23.2
	334 × 906-9	27.0
	334 × 330	27.4
	375-NS14 × 906-9	26.1
Self-fertile isolates	209	35.8
	305	23.0
	307	24.0
	310	24.2
	375	24.3

[a] Each mean is the average of 50 measurements.

their origins and determine if these mated cultures actually hybridize and, if so, determine the antheridial and oogonial isolates.

Isolates were mated on a thin film of clarified tomato-juice agar on coverslips, inverted over Van Tiegham cells, and held at 20°C. The mycelia grew together within 24–30 hours, after which they were placed under microscopic observation. Oogonia began to form within 4–6 hours after mycelial contact. No morphological differentiation of the antheridial or oogonial initials was apparent before they made actual contact. Many copulating gametangia were observed shortly after contact and, on at least one occasion, before penetration had taken place. This developing oogonium was photographed 55 minutes after it was first observed (Fig. 1A). At this time, however, the oogonial initial had already penetrated the antheridium, grown through the interior, and emerged at the summit, where it was rounding into a sphere preparatory to forming the oogonial wall. After 2½ hours the oospore had attained almost full size (Fig. 1B).

The many attempts to trace gametangial initials to their origins were unsuccessful using the cross *P. capsici* × *P. parasitica* var. *nicotianae*. It was difficult to identify the oogonial and antheridial branches at the base of the antheridium, since there was usually an entanglement of mycelia at this point. It was possible, however, to trace the initials to their origins in a mating of two *P. capsici* isolates (209 × 235). Isolate 209, which mated with isolates of the flue-cured strain, supplied the oogonial initial, and 235 supplied the antheridial initial.

DISCUSSION.—Eighty-five per cent of the isolates studied were self-sterile and possibly heterothallic. In *P. capsici* (209) × *P. parasitica* var. *nicotianae* (flue-cured strain) crosses, the phenomenon involved was assumed to be hybridization, although the possibility of chemical stimulation was recognized. This assumption is based on the following experimental and empirical evidence:

1) By using the Van Tiegham cell technique and mating 2 isolates of *P. capsici* (209 × 235), it was determined that 209 supplied the oogonial initial (Fig. 1A) in this cross, thus establishing that 209 was cross-compatible with another isolate. 2) In all crosses involving 209, oospore size and appearance was practically identical (Table 2). This implied a common oogonial parent, which, according to Leonian (9), controls the size of the oospore in mated cultures. This would be 209 since it was common to all these crosses. 3) The resultant oospores were not typical of either species involved. They were small, with hyaline oogonial walls, in contrast to the large, brown type produced in old pure cultures of 209, and were significantly larger than oospores of homothallic black shank isolates. If chemical stimulation were involved, the oospores produced should be typical of one of the species involved, or a mixture of the 2 types if the 2 thalli were mutually stimulated. In the latter case, the oospore size would be intermediate, as was observed, but would contain 2 distinct sizes. When size frequencies of oospores from mated cultures were plotted, the curve had only 1 peak, indicating that 2 distinct size classes were not present. 4) Exchange filtrates did not induce compatible isolates to produce oospores. This constitutes negative evidence of chemical stimulation and indicates that a strain does not excrete into the medium an extracellular substance that induces the complementary

TABLE 3.—*Production of oospores by nonsporulating isolates*[a] *of Phytophthora parasitica var. nicotianae in paired cultures*

Experiment 1

	Mating reaction[b]					
Isolate	306	308	312	313	317	318
306	0	0	0	0	0	I
308		0	0	I	0	I
312			0	0	0	I
313				0	0	II
317					0	0
318						0

Experiment 2

	Mating reaction[b]						
Isolate	325	329	365	376	384	385	416
325	0	0	0	0	0	I	II
329		0	0	0	0	0	I
365			0	0	0		I
376				0	0	0	II
384					0	0	I
385						0	II
416							0

[a] Nonsporulating isolates are those that fail to produce oospores in pure culture.

[b] Number of oospores in an area of 0.042 mm² in the mating zone: 0 = none; I = <1; II = 1–6; III = 7–14; IV = 15–22; V = 23–30; VI = >30.

strain to form oospores. However, this experiment was not conclusive. Hemp-seed water cultures are not very satisfactory for mating purposes; the essential chemical could have been absorbed by the filter; the essential chemical could be unstable; and/or the essential chemical could have been in such low concentration to be ineffectual.

On the foregoing evidence, in pairings of 209 with isolates of the flue-cured strain, the former was considered to supply the oogonial initial, and the latter to supply the antheridial initial. On this basis the isolates of the burley strain, which mated with isolates of the flue-cured strain, are also considered to be oogonial. These assumptions agree with the evidence of Johnson and Valleau (7), who found 33 oogonial and 2 antheridial isolates among 35 of the burley strain studied in Kentucky. These determinations were possible because the antheridial isolates "often produced antheridia in culture when grown alone." The writer was not able to make such a morphological distinction between opposite mating types. Nevertheless, when Johnson and Valleau's antheridial strain (Table 1, isolate 330) was mated with the burley strains used in this study, oospores were formed. Likewise, their oogonial strains (Table 1, isolates 333 and 334) mated with isolates of the flue-cured strain.

Mating studies have shown a basic difference between the burley and flue-cured strains with respect to compatibility type. All isolates of the flue-cured strain were antheridial in that they mated with 209, whereas all collections of the burley strain were oogonial. Johnson and Valleau (7) found 2 antheridial isolates of the burley strain. The absence of antheridial isolates in the collection studied may be a sampling error; even so, the burley strain is considered predominantly oogonial. There is no apparent explanation for this unique geographic distribution of mating types. It might suggest host selectivity, i.e., that burley oogonial isolates have a selective advantage on burley tobacco varieties, whereas flue-cured antheridial isolates have a selective advantage on flue-cured tobacco varieties. Other investigators have attempted to explain the distribution of mating types of other *Phytophthora* species on this basis (6, 8). However, this hypothesis is not supported by experimental evidence: the 2 groups show no differential pathogenic potential on these 2 types of tobacco. Both cultural strains of the black shank fungus occur contiguously only in the Georgia-Florida area; consequently, both mating types are present in this area.

When matings of all possible paired combinations were made among certain nonsporulating flue-cured isolates, the existence of other mating types was evident. Isolates 318 and 416 mated with other flue-cured isolates. This could indicate that sexuality in this organism has a complex genetic base.

Although negative evidence was obtained in this study, chemical stimulation should not be ignored in sexuality studies of the genus *Phytophthora*. Recent studies indicate that chemical stimulation can play a role in oogenesis in the genus *Phytophthora* (8). The fact that little or no differentiation of the female and male gametangia occurs before they make contact indicates that differentiation may require a chemical stimulus.

Many of the isolates studied are self-fertile and possibly homothallic, since they produced oospores in pure culture. Since these isolates did not originate from single oospores, the possibility exists that some of them were heterocaryotic for mating type but were actually heterothallic. However, the single-zoospore isolates from homothallic 375 provided no evidence that the parent culture was heterocaryotic for mating type, since 2 mating types were not recovered. Also, many of the single-zoospore isolates from 375 and other cultures produced zoospores abundantly.

It was postulated that the nonsporulating sectors in normally sporulating cultures arose from mutant nuclei, that single-zoospore isolates from these would yield stable, nonsporulating lines, and that single-zoospore isolates from sporulating sectors would yield stable self-fertile lines. Some self-sterile lines were recovered from 375; however, contrary to expectation, zoospores from sporulating sectors gave a higher percentage of nonsporulating isolates than those from the nonsporulating sectors. Some self-sterile isolates from nonsporulating sectors were unique in that they were compatible with both mating types. Since no simple oogonial isolates were found, it is possible that this unstable condition gives rise only to antheridial isolates. This is analogous to field conditions, since no oogonial isolates have been found among flue-cured isolates.

Measurement of oospores from homothallic isolates (Table 2) agreed closely with those given by Tucker (14) and Johnson and Valleau (7) for *P. parasitica* var. *nicotianae* (average 21–24 μ), but differed significantly from those for the black shank fungus reported from Greece by Kouyeas (8), who considered the "oogonial" size for this organism to be about 38 μ.

Direct observation of developing oogonia supports the interpretation that the oogonial initial penetrates the antheridial initial during oogenesis. Some authors attribute the microscopic observation of this phenomenon to Pethybridge (12), Leonian (9), Narasimhan (10), and Cohen (4). In his original description, Pethybridge did not say that the process was actually observed, although attempts were made. Neither was such a statement made by Leonian, Narasimhan, or Cohen. The latter 3 investigators made direct observations of developing oogonia to determine the male and female thalli, but did not claim to have observed gametangial copulation and subsequent penetration of the antheridium by the oogonial incept.

These studies indicate that the black shank fungus may form intra- or interspecific hybrids. It has been demonstrated that at least some of the oospores resulting in "interspecific" crosses (Fig. 1C) are viable. The perfect stage of this organism may, therefore, be

a source of morphological and pathogenic variability. However, definite proof of heterothallism and homothallism is lacking. The problem of sexuality in the black shank fungus and other *Phytophthora* species cannot be resolved until oospores are germinated in sufficient quantity to permit a study of the controlling genetic factors.

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A NEW STRAIN OF TOBACCO-STREAK VIRUS FROM PEAS[1]

Graciano Patino[2] and W. J. Zaumeyer[3]

SUMMARY

A new virus that produces primary lesions and systemic necrosis on beans was isolated from naturally infected peas collected in southern Idaho. It is described, identified, and compared with several other bean viruses. All 30 bean varieties tested were susceptible. Among 46 plant species tested, 29 from 5 families were found susceptible. The virus was inactivated at 62–64°C in 10 minutes and after 27 hours of storage *in vitro* at about 22°C. It withstood dilution of 1:2000, but not of 1:4000. It was infectious for at least 98 days in dry tissue at room temperature. No evidence of seed transmission was found. On the basis of symptoms produced on beans and other hosts, and of physical properties and certain cross-protection tests, the virus is regarded as a new strain of the tobacco-streak virus. It can be differentiated from the red-node virus by its ability to infect certain hosts not infected by the latter. It differs from the type strain of the tobacco-streak virus in physical properties and host range. The same criteria distinguish it from some other viruses that cause systemic necrosis of bean. The name "pea strain of the tobacco-streak virus" is proposed for the virus described.

In surveying for pea diseases in southern Idaho in June and July, 1956, W. J. Zaumeyer collected several specimens of diseased pea, *Pisum sativum* L., that showed a vein clearing of the leaves. These were sent to Beltsville, Maryland, for determination of the cause. The symptoms were assumed to be initial symptoms of pea streak, caused by a virus widespread in southern Idaho. The specimens were desiccated because temperatures in the greenhouse at that time were too high for pea virus inoculations. The desiccated material, after about 5 months of storage, was inoculated on Pinto U.I. 111[4] bean (*Phaseolus vulgaris* L.) and on pea (*Pisum sativum* L.) plants of several varieties. The peas showed only a slight stem necrosis; the beans showed symptoms of primary and systemic infection similar to those produced by the tobacco-streak virus described as red-node virus

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[4] U.I. indicates that the variety was originated by the University of Idaho.

by Thomas and Zaumeyer (10).

This paper deals with identification of the new virus, describes the symptoms it causes on certain hosts under greenhouse conditions, and compares it with the red-node virus. As reported earlier (7), the new virus is classified as the pea strain of tobacco-streak virus.

MATERIALS AND METHODS.—All investigations were

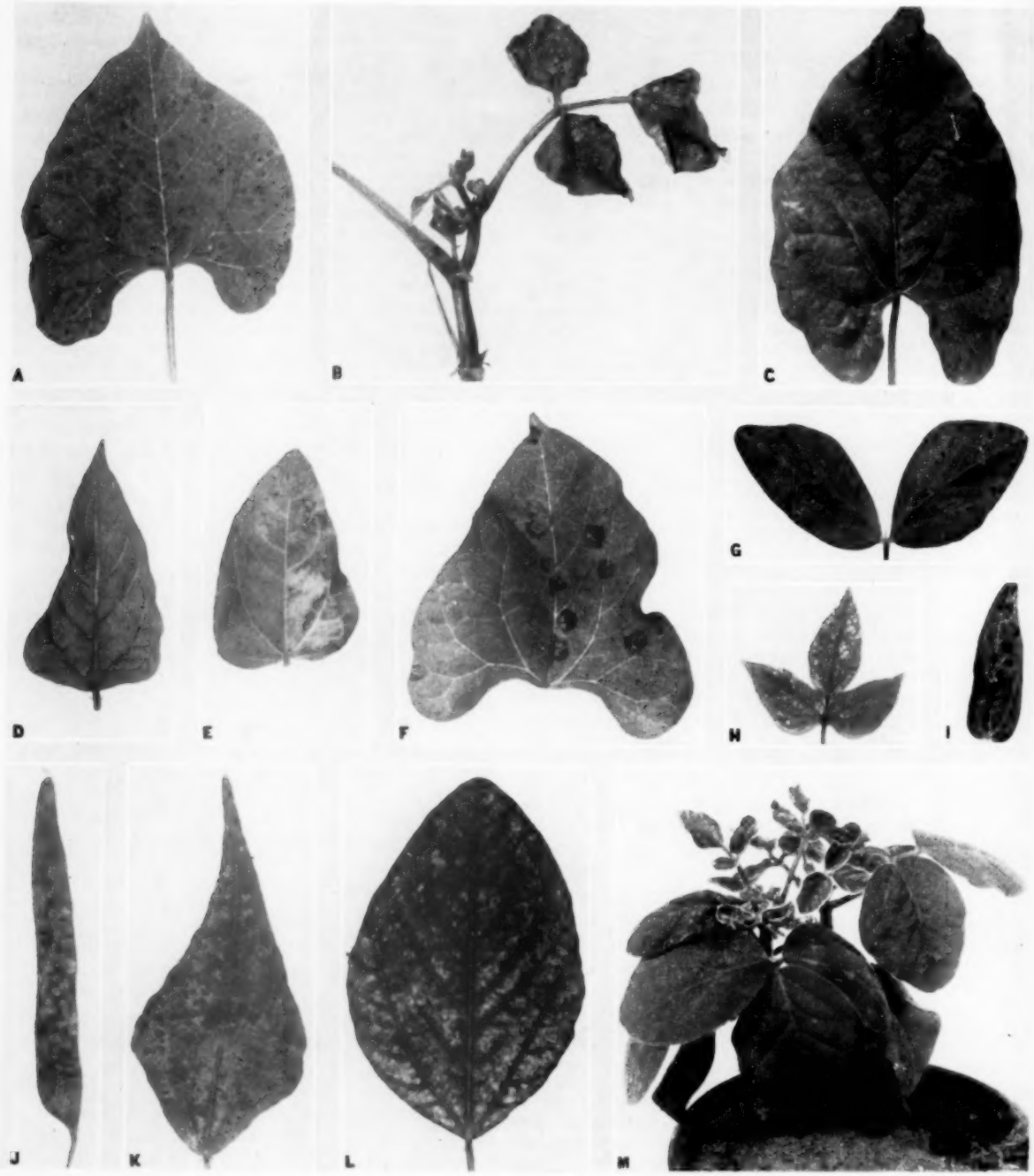

FIG. 1. Symptoms produced by the (new) pea strain of tobacco-streak virus on plants inoculated in the greenhouse. A, F) Necrotic primary lesions on leaves of Pinto U.I. 111 bean. B) Necrosis of stem, petioles, pulvini, and trifoliate leaves of Pinto bean. C) Chlorotic primary ringlike lesions on the leaf of Cherokee bean. D) Veinal necrosis of a trifoliate leaflet of Pinto bean. E, G, I) Necrotic primary lesions on leaves of cowpea var. Iron, *Vicia faba* var. *minor*, and *Phaseolus aureus*, respectively. H) Yellow mottling on trifoliate leaflets of *P. mungo*. J) Necrotic ringlike patterns on pod of Pinto bean. K) Systemic yellow-stipple mottle on leaf of Pinto bean. L) Systemic mottle on leaf of soybean var. Jackson. M) Severe leaf malformation and stunting of soybean var. Roanoke.

carried on under greenhouse conditions at Beltsville at 21–30°C. The diseased peas collected in Idaho were desiccated as described by McKinney (5). The virus was maintained on plants of Pinto U.I. 111 and Stringless Green Refugee beans. When the bean plants were about 9 days old, inoculations were made by lightly dusting carborundum powder 320 on the primary leaves and rubbing them with a cheesecloth pad dipped in juice expressed from infected plants. In all studies of symptoms and host range the juice was diluted about 1:1 with water.

For cross-protection tests, cultures of red-node virus and the McKinney strain of tobacco ring-spot virus were obtained from a collection of desiccated virus-infected plant tissues maintained by the Bean Project at Beltsville. When indistinct symptoms prevented definite determination of the susceptibility of inoculated plants of some species, attempts were made to recover the virus by inoculating plants of one of the above-mentioned bean varieties.

SYMPTOMS AND HOST RANGE.—Studies of symptoms and host range involved 46 plant species. Susceptibility was shown by twenty-nine of them—in the families Leguminosae, Solanaceae, Amaranthaceae, Portulacaceae, and Compositae.

Phaseolus vulgaris L. (common bean). On Pinto U.I. 111 and Stringless Green Refugee varieties, reddish-brown, somewhat ringed, necrotic lesions 1–3 mm in diameter appeared on inoculated leaves 36–48 hours after inoculation (Fig. 1-A,F). Primary lesions were larger when few (Fig. 1-F) than when numerous (Fig. 1-A). Veinal necrosis of inoculated leaves varied in extent. Severely infected leaves usually dropped from the plant. Petioles and stems became necrotic about 5 days after inoculation (Fig. 1-B). Trifoliate leaflets that were systemically infected showed yellow, stipple-like spots (Fig. 1-K), followed by necrosis of the veins and veinlets (Fig. 1-D). In cases of severe necrosis the leaflets were malformed. Mild mottle often developed on young trifoliate leaves also (Fig. 1-K). Small chlorotic rings appeared occasionally on the trifoliate leaves of Pinto U.I. 111, but not on those of Stringless Green Refugee. In cases of severe systemic infection the nodes and pulvini were reddened (Fig. 1-B), and the plants often died. Those that lived were severely stunted. Although most of the pods of infected plants were normal, some developed reddish, ringed patterns (Fig. 1-J) similar to those produced by the red-node virus (10). Symptoms produced by the new virus were in general milder than those produced by the red-node virus.

All 28 of the other inoculated varieties of beans also proved susceptible: Black Valentine, Blue Lake, Bountiful, Brittle Wax, Cherokee, Dark Red Kidney, Florida Belle, Full Measure, Giant Stringless Green Pod, Great Northern U.I. 31, Great Northern U.I. 123, Kentucky Wonder, Kentucky Wonder 814, Kentucky Wonder Wax 765, Kentucky Wonder White, Kentucky Wonder White 780, Landreth Stringless Green Pod, Light Red Kidney, Logan, Michelite, Plentiful, Red Mexican U.I. 34, Rival, Robust, Sensation Refugee 1066, Sure Crop Wax, Topcrop, and Unrivalled Wax. The symptoms were generally similar to those discussed above. On most varieties, the primary lesions appeared as chlorotic rings of various sizes (Fig. 1-C); on Kentucky Wonder White 780 and Rival, the necrotic spots were solid. On Full Measure and Landreth Stringless Green Pod, no primary veinal necrosis was observed. Only Sure Crop and Unrivalled Wax developed epinasty of the simple leaves. Great Northern U.I. 123 was the only variety that did not show yellow stipple mottle.

Cajanus cajan (L.) Druce (pigeon pea). Yellow mottle.

Capsicum frutescens L. (pepper). Distinct yellow mottle. Virus could not be recovered.

Cicer arietinum L. (chick pea). Chlorosis of terminal leaflets and terminal stem streaking.

Datura stramonium L. (jimsonweed). Distinct mottle and numerous small, yellow spots.

Glycine max (L.) Merr. (soybean). Intense yellow mottling (Fig. 1-L) and crinkling of trifoliate leaves. Extreme plant stunting of the variety Roanoke (Fig. 1-M), but not of the varieties Ogden and Jackson.

Gomphrena globosa L. (globe amaranth). Reddish necrotic local lesions.

Lupinus albus L. (white lupine). Small necrotic local lesions, occasional death of inoculated leaves, stem necrosis and terminal killing.

Lupinus angustifolius L. Small necrotic local lesions.

Nicotiana glutinosa L. Small yellow areas on inoculated leaves. Mottling and malformation of upper leaves, followed by development of necrotic spots.

Nicotiana rustica L. Systemic infection manifested as light-green spots on upper leaves.

Nicotiana sylvestris Speg. Numerous small, yellow spots on inoculated leaves, later becoming necrotic. Systemic infection manifested as light-green spotting on upper leaves.

Nicotiana tabacum L. (tobacco var. Samsun). Few small, chlorotic spots on the inoculated leaves. Systemic infection manifested as numerous small, yellow flecks and light-green rings, sometimes concentric, on the upper leaves. Stunting of infected plants.

Petunia hybrida Vilm. (petunia). Elongated necrotic primary lesions. Systemic infection manifested as a yellow mottling along the main veins.

Phaseolus aconitifolius Jacq. (moth bean). Necrotic local lesions.

Phaseolus acutifolius Gray var. *latifolius* Freeman (tepary bean). Very small, numerous, reddish-brown local lesions.

Phaseolus angularis (Willd.) W. F. Wight (adzuki bean). Primary lesions indistinct. Systemic infection manifested as mild mottle of trifoliate leaves.

Phaseolus aureus Roxb. (mung bean). Reddish-brown, ring-like primary lesions with light centers and veinal necrosis (Fig. 1-I). Systemic infection manifested as downward and inward curling of tri-

foliate leaves. Sometimes stem necrosis and death of seedlings.

Phaseolus coccineus L. (scarlet runner bean). Large, spreading primary necrotic lesions and mottle of trifoliate leaves.

Phaseolus mungo L. (urd bean). Small, necrotic primary lesions with slight veinal necrosis. Systemic infection manifested as yellow chlorotic spots (Fig. 1-H).

Pisum sativum L. (pea). Slight stem necrosis on plants of the varieties Early Perfection, Pacemaker, Wilt Resistant Perfection, and Dark Skin Perfection. No infection of Pride and Alaska varieties.

Portulaca grandiflora Hook. Very small, cream-colored, sunken local lesions.

Stizolobium deeringianum Bort. (velvet bean). Slight, reddish veinal necrosis on inoculated leaves. Yellow mottling and puckering of the trifoliate leaves.

Trifolium hybridum L. (alsike clover). Yellow vein banding and few necrotic spots.

Vicia faba var. *minor* Peterm. (bell bean). Brownish, ring-like primary lesions (Fig. 1-G). Slight stem and leaf necrosis.

Vicia sativa L. (vetch). Elongated, reddish-brown local lesions.

Vicia villosa Roth (hairy vetch). Symptoms same as on *V. sativa*.

Vigna sinensis (Torner) Savi (cowpea). Small, reddish, ring-like primary lesions, and slight veinal necrosis (Fig. 1-E). Systemic infection manifested as yellow mottling of trifoliate leaves.

Zinnia elegans Jacq. (zinnia). Symptomless. Virus recovered from inoculated leaves 28 days after inoculation.

No infection was obtained in *Antirrhinum majus* L. (snapdragon), *Astragalus cicer* L. (cicer-milk vetch), *Beta vulgaris* L. (beet), *Brassica oleracea* var. *botrytis* L. (broccoli), *Canavalia ensiformis* (L.) DC. (jack-bean), *Crotalaria spectabilis* Roth (crotalaria), *Cucumis sativus* L. (cucumber), *Cyamopsis tetragonoloba* (L.) Taub. (guar), *Lens culinaris* Medic. (lentil), *Medicago sativa* L. (alfalfa), *Melilotus alba* Desr. (white sweetclover), *Phaseolus calcaratus* Roxb. (rice bean), *Phaseolus lunatus* L. (Lima bean), *Spinacia oleracea* L. (spinach), *Trifolium incarnaturm* L. (crimson clover), *T. pratense* L. (red clover), and *T. subterraneum* L. (sub clover).

PHYSICAL PROPERTIES.—Expressed juice from young systemically infected bean plants was used in studies of thermal inactivation, dilution, and aging *in vitro*. For the last the juice was stored at room temperature (about 22°C). For determining the longevity of the virus in dried tissue, infected bean leaves from greenhouse-grown plants were dried 2–3 days in the open in the laboratory, pulverized, and stored at room temperature in an uncovered beaker protected from direct sunlight. After various periods pulverized tissue was ground with water in a mortar and used to inoculate primary leaves of Pinto U.I. 111 bean. Primary infection of this variety was the measure of virus survival.

TABLE 1.—*Thermal inactivation and tolerance to dilution of the pea strain of the tobacco-streak virus as determined by production of primary lesions on 16 primary leaves of Pinto U.I. 111 variety of bean*

Inactivation[a] temperature (°C)	Total no. lesions Test 1	Total no. lesions Test 2	Dilution	Total no. lesions Test 1	Total no. lesions Test 2
None (control)	[b]	[b]	none	[b]	[b]
58	91	342	1:500	35	26
60	80	160	1:1000	4	16
62	40	36	1:2000	3	6
64	0	0	1:4000	0	0

[a] Time of exposure 10 minutes.
[b] Numerous.

The virus was inactivated in 10 minutes at 62–64°C; it withstood 1:2000 dilution, but not 1:4000 (Table 1).

Virus stored *in vitro* at about 22°C was infectious for 26 hours, but not after 27 hours (Table 2). Virus stored 26 hours produced an average of 2 primary lesions per leaf, compared with about 100 lesions per leaf produced by freshly extracted undiluted juice used as a check.

Virus in dried bean leaves was still infectious at 98 days (Table 2); the maximum survival period was not determined.

SEED-TRANSMISSION STUDIED.—Seeds were harvested from plants of Stringless Green Refugee and Pinto U.I. 111 varieties infected with the pea strain of the tobacco-streak virus, with most pods showing typical virus symptoms. No infection was observed in 147 plants grown from these seeds in the greenhouse.

CROSS-PROTECTION STUDIES.—Mottled leaves of Pinto bean on plants infected with the pea strain of the tobacco-streak virus, together with healthy leaves on uninfected plants of the same age, were inoculated with the red-node virus. Typical primary lesions of the red-node virus were produced on some of the mottled leaves, but not on others. All healthy leaves developed typical red-node symptoms. It is possible that, because of incomplete systemic infection of all the cells of the leaves mottled by the pea strain, protection against infection by the red-node virus was not complete in some cases.

Six *Nicotiana sylvestris* plants systemically infected with the pea strain of the tobacco-streak virus, and 6 healthy plants of the same age, were inoculated with the McKinney strain of tobacco ring-spot virus. Typical tobacco ring-spot symptoms developed in all 12 plants, indicating that the two viruses are unrelated.

DISCUSSION.—The data indicate that the virus described here is related to the red-node virus, a strain of the tobacco-streak virus, even though the inconsistent results of cross-protection tests did not positively indicate a relationship. Both viruses produce similar symptoms on beans and certain other hosts, although the red-node virus produces symptoms that

TABLE 2.—*Tolerance of the pea strain of the tobacco-streak virus to aging in vitro and in dry tissue as determined by production of primary lesions on 16 primary leaves of Pinto U.I. 111 variety of bean*

Aging *in vitro*		Aging in dry tissue	
Hours	Lesions per leaf	Days	Virus recovered
0	100	0	+
16	14	9	+
18	23	16	+
20	5	23	+
21	4	37	+
23	3	44	+
24	4	58	+
26	2	65	+
27	0	73	+
30	0	98	+

are in most instances more severe than those caused by the new virus. In addition, the new virus is distinguished from the red-node virus by its ability to infect adzuki bean, *Nicotiana glutinosa*, petunia, and zinnia, which are not infected by the red-node virus. Conversely, the red-node virus infects cucumber, crotalaria, guar, crimson clover, red clover, and white sweetclover, but the new virus does not.

The physical properties of the two viruses are in rather close agreement (Tables 1, 2, and 3), but vary sufficiently to be distinguished. The thermal inactivation point is 62–64°C for the new virus, and 56–58°C for red-node virus (10). The red-node virus loses infectivity rapidly after storage *in vitro* for 20 minutes at 22°C (10), whereas the new virus is highly infectious after 2 hours and does not show rapid inactivation until after 18 hours. Thirty minutes after extraction, the red-node virus produced an average of 1 lesion per leaf, whereas the new virus produced about 100. The red-node virus was infectious after storage *in vitro* for 24 hours, but not after 48 hours; the new virus was infectious after 26 hours, but not after 27 hours. Neither virus lost its infectivity in dried leaf tissue in 30 days at room temperature, and the new virus was still infectious at 98 days. The red-node virus withstood dilution of 1:500 but not 1:1000; the new virus was infectious at 1:2000 but not at 1:4000.

The type strain of tobacco-streak virus in juice extracted from infected beans is inactivated in 10 minutes at 54–56°C, and its dilution end point is 1:500–1:1000 (10). From another host, this virus is reported to have a thermal inactivation point of 52–54°C (1), and a dilution end point of 1:20–1:30 (8). Its inactivation was rapid within 20 minutes after extraction, and complete after storage for 1–24 hours at room temperature (10). Beet, crimson clover, cucumber, guar, red clover, snapdragon, spinach, and white sweetclover have been infected by the type strain of the tobacco-streak virus (8), but not by the new virus. It is apparent that the virus described here is closely related to the red-node strain of the tobacco-streak virus, but that it can be differentiated from it and the type strain on the basis of host range and physical properties (Table 3). The data suggest that the virus should be referred to as the pea strain of the tobacco-streak virus.

Systemic necroses of beans are caused not only by the viruses of red node and tobacco streak, but also by the viruses of tobacco ring-spot (8), the bean strain of tobacco ring-spot (3), stipple streak (11), alfalfa N (6), yellow dot (9), alfalfa yellow mosaic (13), and the necrotic-lesion strain of yellow bean mosaic (14). Through differences in physical properties and host range, the pea strain of the tobacco-streak virus can be distinguished from the tobacco ring-spot virus, from the bean strain of the latter virus, and from the stipple-streak virus. The tobacco ring-spot virus (8) and the bean strain of this virus

TABLE 3.—*Seven viruses that cause systemic necrosis of bean: reaction of certain species; and inactivation and dilution end points*

	Host reaction[a]											End points	
Virus	Antirrhinum majus	Capsicum frutescens	Cucumis sativus	Medicago sativa	Meliotus alba	Nicotiana glutinosa	Petunia hybrida	Phaseolus angularis	Pisum sativum	Trifolium incarnatum	Trifolium pratense	°C[b]	Dilution
Pea strain of tobacco streak	—	+	—	—	—	+	+	+	+	—	—	62–64	1:2000–1:4000
Red node (10)			+	—	+	—	—	—	+	+	+	56–58	1:500–1:1000
Tobacco streak (8)	+	+	+		+	+	+		+	+	+	54–56	1:500–1:1000
Bean strain of tobacco ringspot (3)		—	+		—	+	+	+	+	+	+	65–70	ca. 1:10,000
Yellow dot (9)		—	+	+	+	+		+	+	+	+	62–64	1:2000–1:3000
Alfalfa yellow mosaic (13)	+	—	—	+	+	+	—		+	+	—	65–70	1:4000–1:5000
Necrotic-lesion strain of yellow bean mosaic (14)			—	—	+	—		—	—	—	—	60–62	1:2000–1:4000

[a] + = infection; — = no infection.
[b] Exposure for 10 minutes.

(3) are inactivated at 65–70°C, and the stipple-streak virus at 85–90°C (11). The dilution end point is about 1:10,000 for the two ring-spot viruses (3, 8), and 1:1,000,000 for the stipple-streak virus (11). Aging *in vitro* at room temperature inactivates tobacco ring-spot virus in 3–4 days, and the bean strain of that virus in 15 days. The two ring-spot viruses infect beet, cucumber, crimson clover, guar, and spinach (3), none of which were infected by the pea strain of tobacco-streak virus.

The thermal inactivation point of the alfalfa N virus is considerably lower than that of the virus described here, and 10–15°C lower than the inactivation points reported for other strains of the alfalfa mosaic virus (6). The thermal inactivation points of the strains of alfalfa mosaic virus are 53–70°C (13).

Some of the physical properties of the viruses of yellow dot, alfalfa yellow mosaic, and the necrotic-lesion strain of yellow bean mosaic are in rather close agreement with those of the pea strain of the tobacco-streak virus, although the host ranges differ (Table 3). Yellow dot virus produces no pod symptoms on beans, whereas the new strain of tobacco-streak virus produces severe necrosis (Fig. 1-J).

The pea strain of the tobacco-streak virus produces a mild stem streaking on peas, but there is no relationship with the pea streak viruses described by Zaumeyer (12), Chamberlain (2), and Hagedorn and Walker (4). Only the virus reported by Chamberlain is infectious to beans. It produces both primary and systemic necrotic symptoms, differs greatly in physical properties from those reported here, is inactivated at 78–80°C, has a dilution end point of about 1:1,000,000, and withstands aging *in vitro* for 41 days, but not 44 (2).

TECHNICAL DESCRIPTION OF NEW VIRUS STRAIN.—*Host reactions.*—Primary necrotic lesions produced on Pinto U.I. 111, Stringless Green Refugee, and other bean varieties 36–48 hours after inoculation, followed by stem and petiole necrosis, slightly reddened nodes, mild mottling of young trifoliate leaves, and necrotic-ring patterns on pods. Primary symptoms consisted of chlorotic rings and veinal necrosis on most varieties, and of primary and systemic yellow or light-green spots or rings on tobacco. Infectious to 29 of 46 plant species tested in the greenhouse.

Transmission.—Readily transmitted by inoculation with expressed plant juice rubbed on leaves. Apparently not seed-transmitted.

Physical properties.—Inactivated in bean leaf juice in 10 minutes at 62–64°C. Dilution end point in fresh plant extract from infected bean between 1:2000 and 1:4000. Withstood storage *in vitro* at 22°C for 26 hours, but not for 27 hours. Infectious after storage in dried bean leaves at room temperature for at least 98 days.

Differences from type species.—No necrotic symptoms on *Nicotiana tabacum* and *N. rustica*. No infection of beet, crimson clover, cucumber, guar, red clover, snapdragon, spinach, and white sweetclover. Thermal inactivation end point 8–10°C higher than for the type strain of the tobacco-streak virus. Higher dilution end point.

Type locality.—Southern Idaho.

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A LATENT VIRUS OF CALIFORNIA ARTICHOKES[1]

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SUMMARY

A latent virus, found widespread in artichoke plantings in California, can infect various species of *Compositae*, *Chenopodiaceae*, *Solanaceae*, and other families. *Chenopodium amaranticolor* is a good local-lesion host plant. The virus is easily transmitted by mechanical inoculations with the use of an abrasive. From artichoke leaves, the crude virus is readily inactivated unless a reducing agent such as sodium sulfite is added during extraction. Inactivation occurs less readily in inoculum from *Zinnia elegans*, and still less so from *Nicotiana clevelandii*. The artichoke latent virus remains active *in vitro* at room temperature for 48 hours in extract prepared in presence of sodium sulfite; it stands immersion for 10 minutes in a water bath at 65°C, but not at 70°C; activity is greatly reduced by dilution to 5^{-3}, though some is still detectable at 5^{-5}. *Myzus persicae* is a vector, but vegetative propagation seems mainly responsible for perpetuation of the virus in commercial plantings. A description of the artichoke latent virus is given under the name of *Marmor cynarae*.

In routine tests on possible relationships of various weed and crop plants to virus diseases of sugar beet, a previously undescribed latent virus was found to be widespread in artichoke (*Cynara scolymus* L.) in California. An investigation was made of the possibility that this virus could be related to a latent virus that attacks sugar beet (*Beta vulgaris* L.) in England (7), or to the dodder latent virus, which also attacks sugar beet (2).

MATERIALS AND METHODS.—Most of the tests with the artichoke latent virus were carried out by mechanical inoculation. In all routine tests, the inoculum was prepared by crushing leaves of artichoke or other host plants in a sterilized mortar in the presence of about 5 parts of 0.02*M* phosphate buffer at pH 7 to which sodium sulfite was added to reach a concentration of 0.02*M*. In many tests, sodium sulfite alone was used as a diluent.

Activity of the artichoke latent virus in the various types of inocula was determined by mechanical inoculation on *Chenopodium amaranticolor* Coste & Reyn., a host plant that develops local lesions on inoculated leaves. Seedlings of this species were grown in pairs in 6-in. pots. In routine tests they were used at the stage of 4 leaves or more; in tests comparing several virus preparations they were used when 6–8 leaves were well developed. Most of the property tests were carried out by the half-leaf method; otherwise the whole leaf was used. Latin square or randomized plot designs were used for distribution of the treatments on the plants. Carborundum was sprinkled on leaves before inoculation.

C. amaranticolor is a good local-lesion test plant for the artichoke latent virus, but it does not serve as an indicator, since many other viruses cause similar local lesions on its leaves. Those caused by the latent virus are generally slow to appear. When necessary for identification, plants of wild lettuce (*Lactuca scariola* L.) or of *Nicotiana clevelandii* Gray were also used.

SURVEY FOR ARTICHOKE LATENT VIRUS IN COMMERCIAL PLANTINGS.—After finding a latent virus in apparently healthy artichoke plants in the field, it became of interest to determine its incidence. Several artichoke plantings were sampled in various California counties—4–12 plants from each of 11 plantings in Monterey, San Luis Obispo, Santa Barbara, Santa Cruz, San Mateo, and Marin counties. One leaf was taken from each of a number of plants chosen at random, and inoculum prepared from the individual leaves was inoculated separately on *C. amaranticolor* test plants. The virus was present in all of the 65 leaves tested. Although the sampling of plantings and plants was relatively small, the results indicate that the latent virus is quite general in these areas.

HOST RANGE.—All types of the Globe artichoke grown in California apparently carry the latent virus. It was also recovered from plants of the type commonly referred to as brown artichoke.

Tests were carried out in the greenhouse to determine the host range of the artichoke latent virus, and to find a good indicator plant. A minimum of 5 plants were tested from each of several species of *Compositae*, *Leguminosae*, and *Solanaceae*, as well as other families. Inoculum was obtained from apparently healthy artichoke plants and applied mechanically by routine methods. Since the virus could also be latent in some other species, all inoculated plants were tested for virus about 20–30 days after inoculation. In these tests inocula were prepared separately from each plant, both from inoculated leaves and from leaves of the young growth, and applied on *C. amaranticolor* test plants. Species from which the latent virus was recovered only from inoculated leaves include *Beta vulgaris* L., *Chenopodium album* L., *C.*

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amaranticolor Coste & Reyn., *C. ambrosioides* L., *C. capitatum* (L.) Asch., *C. murale* L., *Lamium amplexicaule* L., *Malva parviflora* L., *Nicotiana glutinosa* L., *N. tabacum* L., *Spinacia oleracea* L., and *Tetragonia expansa* Murr. Species systemically infected by the latent virus include *Cynara scolymus* L., *Helianthus annuus* L., *Lactuca scariola* L., *Nicotiana bigelovii* S. Wats., *N. clevelandii* Gray, *Phaseolus vulgaris* L., *Senecio vulgaris* L. and *Zinnia elegans* Jacq. No symptoms were observed on, or virus recovered from, inoculated pokeweed (*Phytolacca americana* L.), cowpea (*Vigna sinensis* (Torner) Savi), and several other species.

SYMPTOMS.—*Artichoke.*—No symptoms have been noted in infected field artichoke plants or in infected seedlings maintained in a greenhouse at about 80°F or slightly higher. The latent virus has been recovered from old and young leaves of artichoke plants, as well as from the bracts of the flower heads.

Chenopodium amaranticolor.—This species develops local lesions on inoculated leaves 7–10 days after inoculation. The lesions appear as minute chlorotic spots, later showing a light-brown center surrounded by a chlorotic area; they are usually circular, but may be elongated if occurring on a vein. Young lesions are about 1 mm in diameter. They enlarge with age, but show no tendency to coalesce. Old lesions are 2–3 mm in diameter and consist of a light spot surrounded by a reddish area.

C. amaranticolor can be used as a test plant for the artichoke latent virus at almost any age, but results are best with vigorously growing plants with 6–8 well-developed leaves. When 6 middle leaves on each of 10 plants were inoculated with virus preparations from artichoke leaves, the number of lesions per leaf, from bottom to top, was 229, 373, 430, 478, 601, and 305 in one test, and 286, 383, 625, 664, 619, and 376 in a second.

To show that the virus, and not a toxic effect of the artichoke extract, produced the lesions obtained on *C. amaranticolor*, inoculum prepared from these lesions was applied to other *C. amaranticolor* plants. The lesions were punched out with a cork borer and crushed in a mortar in the presence of 0.02*M* sodium sulfite. Local lesions produced on leaves of *C. amaranticolor* with this inoculum were identical to those obtained with inoculum from infected artichoke plants. Furthermore, the same type of lesion was obtained when test plants of this species were inoculated with preparations of the latent virus from such other host plants as zinnia, *Nicotiana clevelandii*, and other susceptible species.

Systemic symptoms are not shown by *C. amaranticolor* plants infected with the artichoke latent virus. The virus could not be recovered from the young, upper leaves of plants whose lower leaves were covered with local lesions. Further, these lesion-free upper leaves were susceptible to infection, as shown by the development of local lesions when they were inoculated with the virus. Thus, this host can be used more than once, although old plants are less susceptible to infection.

Nicotiana clevelandii.—Infected plants of this species did not show very definite symptoms of disease, although a faint, indefinite mottle could sometimes be noted.

Chenopodium murale.—Sowbane plants developed local rings in leaves inoculated with the artichoke latent virus. These lesions took longer to form than did lesions on *C. amaranticolor*, but were larger and had a concentric pattern. Leaves with lesions were often shed by the plants. The virus did not become systemic.

Chenopodium ambrosioides.—This species formed small, reddish local lesions on leaves inoculated with the artichoke latent virus. Systemic symptoms were not noted, nor could the virus be recovered from the young growth of plants with local lesions.

Lactuca scariola.—Infected greenhouse plants showed yellowish spots in inoculated leaves, and irregularly shaped chlorotic areas developed in young leaves following systemic invasion of the host.

Phaseolus vulgaris.—Bean plants of the varieties Bountiful, Pinto, and Small White showed chlorotic local lesions in their primary leaves 7–10 days after inoculation. Systemic symptoms, when present, appeared as a chlorosis along the veins.

Sugar beet.—Yellowish areas were noticed on sugar beet leaves inoculated with the artichoke latent virus. Virus was recovered from inoculated leaves but not from young uninoculated leaves.

TRANSMISSION TESTS.—The artichoke latent virus is perpetuated through vegetative propagation in cuttings from infected plants. Spread in the field by other means has not been demonstrated.

An attempt was made to recover the artichoke latent virus from artichoke seedlings set in the field among diseased plants. None was recovered from 5 potted seedlings that had been growing 4 months about 20 in. from infected field plants.

A few weed species growing in artichoke plantings were tested for the latent virus, but without positive results.

Seed.—About 50 artichoke seedlings, from a commercial seed source, that very likely had been produced from plants carrying the virus were grown in the greenhouse, and found virus-free when tested by inoculation of their leaf extracts on the leaves of *C. amaranticolor*.

Vector.—In preliminary tests, the aphid *Myzus persicae* (Sulz.), fed on infected artichoke shoots, did not transmit the latent virus to zinnia test plants. In later tests, the same insect, fed on infected *Nicotiana clevelandii* plants, transmitted the latent virus to artichoke, *N. clevelandii*, and *Lactuca scariola* seedlings.

In other tests, *Aphis fabae* Scop. and *Capitophorus braggi* (Gillette), fed on infected artichoke shoots, did not transmit the latent virus to artichoke, *N. clevelandii*, or zinnia seedlings.

Mechanical transmission.—The artichoke latent

TABLE 1.—*Results of inoculation tests on Chenopodium amaranticolor to determine the effect of diluents on the activity of the artichoke latent virus preparations*

Diluent added to inoculum[d]	Number of lesions obtained on 16 half-leaves with inoculum extracted in presence of the indicated diluent			
	1[a]	2[a]	3[b]	4[c]
None (undiluted juice)	0	19	34	554
Water	0	120	54	552
0.02*M* sodium sulfite	327	424	137	408
0.02*M* phosphate buffer	31	76	56	390
Mixture of buffer plus sulfite	313	803	105	564

[a,b,c] Inoculum from artichoke, zinnia, and *Nicotiana clevelandii* leaves, respectively.
[d] All diluents added to inoculum before grinding in the proportion of 5 to 1.

virus is easily transmitted by the routine procedures in mechanical inoculation that include use of a reducing agent and an abrasive.

Inoculum was slightly better from young artichoke leaves than from old leaves. In 3 tests, inocula from young leaves applied to 6 half-leaves of *C. amaranticolor* produced 122, 178, and 703 lesions; inocula from old leaves on the other half-leaves produced, respectively, 140, 93, and 286 lesions.

CROSS-INOCULATION TESTS WITH THE ARTICHOKE LATENT AND LETTUCE MOSAIC VIRUSES.—Because of a certain similarity between artichoke latent and lettuce mosaic viruses in host range and certain properties, a few attempts were made to compare them by cross-inoculation.

About 20 seedlings of each of 2 lettuce varieties, Eiffel Tower Cos and Bibb, were inoculated mechanically with the artichoke latent virus, and 20 artichoke seedlings were inoculated mechanically with the lettuce mosaic virus. In addition, 10 artichoke seedlings were inoculated with the lettuce mosaic virus by means of *Myzus persicae*.

The artichoke latent virus did not induce any symptoms in the 2 lettuce varieties, nor could the virus be recovered from the inoculated plants. Also, the lettuce mosaic virus could not be recovered from the inoculated artichoke plants.

PROPERTIES OF THE VIRUS IN PLANT EXTRACT.—*Loss of activity during extraction.*—Early experiments indicated that latent virus from artichoke leaves easily loses its activity unless a reducing agent is added during extraction. A few tests were then carried out to compare the effects of common diluents: water, 0.02*M* sodium sulfite, 0.02*M* phosphate buffer at pH 7, and a mixture of the last 2. Artichoke leaves were cut in small pieces and well mixed. A number of comparable samples were prepared and each was mixed with the diluent in a mortar in a 1:5 proportion. The samples were crushed and strained before inoculation. Undiluted juice was obtained by grinding leaves without diluents. Samples from other source plants were prepared in the same way.

The activity of the preparations was measured on half-leaves of *C. amaranticolor*. Table 1 shows the results of 4 tests with 3 sources of inoculum. If sodium sulfite was not added during extraction, loss of activity in the virus preparation was great with artichoke leaves, less noticeable with zinnia leaves, and not noticeable with *Nicotiana clevelandii* leaves. Water or phosphate buffer alone had a slight beneficial effect. A mixture of buffer and sodium sulfite was equal to or slightly better than sodium sulfite alone.

Virus preparations extracted from artichoke leaves in the presence of water or phosphate buffer turned brownish rather quickly, whereas those crushed in the presence of sodium sulfite tended to remain greenish longer. Preparations from zinnia leaves showed similar, but less marked, color changes, and preparations from *Nicotiana clevelandii* leaves showed no color changes.

Aging in vitro.—The first test carried out with undiluted artichoke juice to determine the resistance of the latent virus to aging *in vitro*, gave totally negative results. Later, aging tests were carried out with virus preparations from artichoke, zinnia, and *N. clevelandii*, comparing juices extracted in the presence of water with juices extracted in the presence of a mixture of buffer and sodium sulfite. The diluents were added in a 1:5 proportion. The results of the aging tests are presented in Table 2. The loss of activity of the latent virus *in vitro* at room tem-

TABLE 2.—*Results of inoculation tests on Chenopodium amaranticolor to determine the resistance of the artichoke latent virus to aging in vitro*

Aging of inoculum (hours)	Total number of lesions obtained on 5 leaves with inoculum obtained from the indicated hosts, treated and aged as indicated					
	Artichoke		*Nicotiana clevelandii*		*Zinnia elegans*	
	Buffer & sulfite	Water	Buffer & sulfite	Water	Buffer & sulfite	Water
0	298	58	154	186	53	13
6	56	0	150	6	57	2
12	31	0	50	10	52	8
24	7	0	17	0	26	1
48	0	0	29	0	7	0
96	0	0	0	0	0	0

TABLE 3.—*Results of inoculation tests on Chenopodium amaranticolor to determine the thermal inactivation point of the artichoke latent virus*

Temperature (°C)	Number of lesions obtained on 5 leaves when the inoculum was treated at the indicated temperature for 10 minutes in 6 tests[a]					
	1	2	3	4	5	6
Untreated	880	165	89	69	114	194
50	66	104	4	83	53	152
55	11	5	1	32	19	96
60	0	2	1	8	13	20
65	0	0	0	0	1	2
70	0	0	0	0	0	0

[a]Tests 1–3, inoculum from artichoke; tests 4–6, from *Nicotiana clevelandii*.

perature was rather rapid when the preparations were not extracted in the presence of the mixture of buffer plus sodium sulfite. After 6 hours, activity was greatly reduced and disappeared within 24 hours. When the mixture of buffer plus sodium sulfite was added, activity was maintained at a higher level for a longer period. After 48 hours there was still active virus in some preparations. Preparations aged for 96 hours gave negative results.

Thermal inactivation point.—Artichoke latent virus preparations used in thermal inactivation determinations were extracted in the presence of a mixture of sodium sulfite and phosphate buffer, added at 1:5 before the leaves were ground in a mortar. The results (Table 3) show a great reduction in activity in samples from artichoke treated 10 minutes at 55°C, although preparations treated at 60°C produced some lesions on *C. amaranticolor* test plants. At the lower temperatures tested, inoculum from *Nicotiana clevelandii* was less affected than inoculum from artichoke.

The thermal inactivation point of the artichoke latent virus appears to be near 60°C, but some activity was occasionally noted after treating at 65°C.

Dilution end point.—A series of dilution tests were carried out with the artichoke latent virus. The inocula, prepared at a concentration of 1 in 5, were diluted in 5 steps up to 5^{-5} with sodium sulfite or a mixture of sodium sulfite and buffer. The results (Table 4) indicate that the dilution end point is near the highest dilution tried, which showed only traces of activity.

ELECTRON MICROSCOPY AND SEROLOGY.—Specimens for electron microscopy of the artichoke latent virus were prepared by routine methods from artichoke, Pinto bean, zinnia, and tobacco virus sources, as well as from healthy control plants. All definite particle types observed in the virus preparations were also present in the controls.

Preliminary serological tests were made with antiserum from rabbits that had been injected with antigen from artichoke carrying the latent virus. Routine procedures were followed in preparing the antiserum. After absorption it was tested against virus antigen preparations from infected artichoke and zinnia. Results of the precipitin tests were negative. The failure may be due to the fact that the virus preparations used to inject the rabbits were from artichoke, which is now known to provide unstable virus extracts.

NAME AND DESCRIPTION OF THE LATENT VIRUS.—The known characteristics of the artichoke latent virus coincide with those of viruses belonging, according to Holmes' system of classification (5), to the genus *Marmor*. A comparison of the host range and other properties of this virus with those of the several other species of *Marmor* reported in the literature failed to reveal any with which it fully agrees. The artichoke latent virus is, therefore, thought not to have been described previously, and the name *Marmor cynarae* is proposed, with the following description:

Marmor cynarae sp. nov.

Common name: artichoke latent virus. Virus found latent in commercial artichoke field plants (*Cynara scolymus* L.); found to be latent and systemic in *Zinnia elegans* Jacq., *Senecio vulgaris* L., and other plants in the greenhouse; it causes indefinite mottling symptoms in *Nicotiana clevelandii* Gray, and *N. bigelovii* S. Wats.; it causes a mottle in wild lettuce (*Lactuca scariola* L.), but does not infect the cultivated species, *L. sativa* L. Local increase of the virus occurs in *Chenopodium amaranticolor* Coste & Reyn., *C. ambrosioides* L., *C. murale* L., and several other species. *C. amaranticolor* is a good local-lesion plant.

Virus transmitted by *Myzus persicae* (Sulz.). Perpetuation of the virus through vegetative multiplication of artichoke plants. Seed transmission improbable; easily transmitted by mechanical inoculation with the use of an abrasive. Inoculum from artichoke leaves is rapidly inactivated if a reducing agent is not added during extraction. Virus is inactivated *in vitro* at room temperature in 96 hours; withstands dilution of 5^{-5} and 10 minutes at 65°C, but not at 70°C.

Descriptive habitat: widespread in commercial artichoke plantings in California.

DISCUSSION.—Since commercial artichoke plantings are obtained by vegetative propagation, it is not surprising that a latent virus is present in practically all field plants. It would not be too surprising if even more than one virus were being carried in old clones of artichokes without apparent effects.

TABLE 4.—*Results of inoculation tests on Chenopodium amaranticolor to determine the dilution end point of the artichoke latent virus*

Dilution of inoculum	Number of lesions obtained on leaves in 5 tests[a]				
	1	2	3	4	5
5-1	229	427	87	206	725
5-2	206	223	175	98	334
5-3	94	26	29	25	51
5-4	4	12	6	5	6
5-5	2	2	4	1	3

[a]Tests 1–3, with inoculum from artichoke; 4, from zinnia; and 5, from *Nicotiana clevelandii*.

The importance of the artichoke latent virus appears negligible for this crop at present, since the plantings where it occurs seem to yield satisfactorily. It is possible, however, that comparable virus-free artichoke plantings would yield better. It must also be taken into account that, even if the latent virus is carried without causing appreciable injury to the plant, its universal presence in the planting might greatly increase the risk of severe losses from certain double virus infections.

So far no connection has been found between the artichoke latent virus and a virus disease of another economic crop. The presence of the virus in all artichoke plantings, however, might represent a potential virus source for other susceptible crops.

The fact that the latent virus was present in all plants tested in commercial artichoke plantings indicates that the virus must be easily transmitted under California field conditions or that the vegetative material propagated in the state was obtained originally from the same infected sources. At present, propagation with offshoots from infected plants is probably the main factor responsible for the wide distribution of the virus.

The possibility has been considered that the artichoke latent virus could be related to the lettuce mosaic virus, perhaps representing a derivative that has become adapted to the artichoke plant. Both affect plants of the Compositae and induce mosaic symptoms in wild lettuce. Their physical properties are not greatly different, and the activity of both viruses is prolonged in the presence of sodium sulfite (1). Both viruses cause local lesions on *Chenopodium amaranticolor*, although those caused by the lettuce mosaic virus appear sooner after inoculation; in addition, lettuce mosaic virus becomes systemic in this host plant, whereas the artichoke latent virus does not. Cross-inoculation tests failed to show that the two viruses are related.

Another virus, the properties of which somewhat resemble those of the artichoke latent virus, is the dodder latent virus (2). This virus, however, causes marked systemic symptoms in sugar beet, tomato, and *Chenopodium murale;* it produces local lesions in pokeweed (*Phytolacca americana* L.) The artichoke latent virus was recovered from inoculated leaves of sugar beets, but no systemic symptoms were noticed in this species nor was the virus recovered from new growth. The artichoke latent virus did not cause symptoms in tomato, and induced only local lesions in *C. murale*. No local lesions resulted when the artichoke latent virus was inoculated on pokeweed plants.

The artichoke latent virus can be readily distinguished from the beet latent virus (7) by its higher thermal end point and by its failure to cause local lesions on cowpea leaves.

The *in vitro* properties of highly stable viruses do not vary greatly in plant extract from different host species (4, 6). The properties of unstable viruses also should not vary in case of purified preparations, but that is not always so when they are determined in crude extract from different host plants. This was shown to be the case for the tomato spotted wilt virus (3) and was noticed by the writers in property determinations of the artichoke latent virus. These differences are probably not inherent to the virus itself, but result from the variation in the inhibitory systems found in extract from different plant species, the action of which may be enhanced by heat treatment or aging.

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THE CAPACITY AND PERIOD OF MAXIMUM PRODUCTION OF SPORIDIA IN CRONARTIUM RIBICOLA[1]

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SUMMARY

A quantitative method of measuring teliospore germination was based on the ability of a telial column of *Cronartium ribicola* to cast sporidia. The beginning, crest, and capacity of sporidium production were determined from the individual detached telial columns of known size and age formed both at constant temperatures and out-of-doors. Time intervals were determined by using the clocks of rotating drum-type hygrothermographs. A representative telial column 1,330 × 105 μ, 10 days old, from *Ribes aureum* Pursh held at 16°C, cast 4,930 sporidia. Casting began 8.5 hours after subjection of the column to 100% humidity. The peak of production began after 14 hours and continued through the 45th hour. Complete exhaustion of the column took 70 hours from the time of subjection. A telial column 770 × 73.6 μ, 7–9 days old, also from *R. aureum* but kept out-of-doors, cast 2,954 sporidia. Casting began during the 7th hour; peak of production occurred between the 11th and 30th hours. Forty-three hours after subjection to 100% humidity the column stopped casting sporidia. Artificial light had no effect on germination of teliospores and subsequent casting of sporidia.

INTRODUCTION AND REVIEW OF LITERATURE.—The measure of the viability of teliospores of *Cronartium ribicola* Fischer is the number of sporidia produced by each telium.[3] Infection of white pines by this fungus depends largely on favorable microclimatic conditions between the production of sporidia on *Ribes* spp. and their penetration of pine needles. The time required for teliospores to begin germination and the time required for maximum production and dissemination of sporidia are of paramount importance in infection studies. In this study a quantitative method of measuring teliospore germination was based on the ability of a telial column to cast sporidia.

York and Snell (9), while conducting inoculation experiments with *Pinus strobus* L., found that sporidia became fully developed 5–6 hours after dry teliospores from leaves of cultivated *Ribes nigrum* L. were brought under favorable conditions. Spaulding and Rathbun-Gravatt (6) found that fresh teliospores that were formed out-of-doors germinated and reached maximum sporidium production in 12 hours at 13–21°C. Their germination tests were conducted by floating the telial columns on water. They further showed that, if telia-bearing ribes leaves were first kept air dried or kept out-of-doors exposed to wind and rain, the time necessary for germination and production of sporidia increased directly with time of such storage.

Hirt (3), using telial columns from naturally infected *R. nigrum*, found that total time from beginning of teliospore germination to casting of the first sporidium was, in a representative case, 11 hours and 23 minutes. Telia were germinated on 1.5% water agar at 11°C. He found the optimum temperature range for telial germination to be 12–18°C, with minimum and maximum temperatures at 0–1° and 21°C. He further found (4) that, under controlled conditions, the average number of spores cast was the same from the 6th hour through the 24th hour, at which time he concluded his experiments. In these studies he suspended heavily infected leaves of *R. nigrum* over water agar in Petri dishes and took the average count of spores in 4 microscope fields each hour. Tests were conducted at 18°C and 100% relative humidity. Van Arsdel, Riker, and Patton (8) reported that 42–48 hours were needed to form and germinate sporidia from teliospores formed at a constant temperature of 16°C.

The present study was undertaken because there were discrepancies in the literature on the time period required for sporidium production and because a quantitative method of measuring telial viability was needed.

MATERIALS AND METHODS.—Aeciospores used as original inoculum were collected from cankers on sugar pine (*Pinus lambertiana* Dougl.) from 2 different areas in northern California. To reduce the chance of contamination by *Cronartium occidentale* Hedgcock, Bethel & Hunt, urediospores from plants growing in the wild were not used. In addition all ribes used were protected from contamination by urediospores and aeciospores of this "pinyon blister rust" fungus. This was accomplished by keeping the inoculated plants in the closed climate chambers of the laboratory or of the greenhouse. Inasmuch as neither disease occurs naturally on ribes in the Berkeley area, it could be assumed that the only chance of contamination would be from urediospore material brought in from the Sierra Nevada.

Ribes were inoculated simply by atomizing aeciospores suspended in 1:10,000 "Tween 60" onto the

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[3] Throughout this paper the term telia or its singular, telium, is used to denote the sorus in which the teliospores are borne. In the genus *Cronartium* the catenulate teliospores form long columns, and for this reason the two terms "telia" and "telial columns" are used interchangeably.

leaves, then holding the plants at 100% humidity and 15–20°C for 24 hours. Urediospores also were used in this same manner; these spores were obtained from ribes originally inoculated with aeciospores and held in 25°C greenhouse cubicles.

The telial columns used were of various lengths, diameters, ages, and formation temperatures. *Ribes aureum* Pursh and *R. nevadense* Kell. were the host plants. All telial columns used were first examined under a compound microscope to ensure that no pregermination of teliospores had taken place. At the same time their length and diameter were measured.

The majority of the tests were conducted in the specially designed climate control mycrotron of the Department of Plant Pathology, University of California, Berkeley.

For studying rate and capacity of sporidium production, the clocks of rotating drum-type hygrothermographs, Friez Model 594, were used (Fig. 1). The diameter of the clock matches the diameter of the bottom of a 100-mm Petri dish.

Telial columns were attached by their basal end to water agar blocks, which were then placed on the under side of Petri-dish lids. Special care was taken to ensure that the telial columns were not curved and that they hung in a vertical position over a water agar surface in the bottom half of the dish. The pH of the agar was adjusted to 2.0 to prevent primary sporidia from germinating and confusing the total count by producing secondary, tertiary, and quaternary sporidia. (The formation of these latter spore stages, common in *C. ribicola*, will be reported in a subsequent paper.) Fifteen to 20 drops of 1*N* HCl per 20 ml of 2% water agar brought pH down to the level desired.

The bottom half of the dish was attached to the clock drum with small pieces of modeling clay, and the lid was suspended over the bottom half by allowing its edges to rest on the ends of 4 wooden pegs placed around the clock drum. The pegs were long enough to allow the lid to fit down over the bottom half of the dish but just clear it. The lid, containing the telial columns, thus remained stationary while the bottom half rotated with the clock, leaving a circle of cast sporidia on the layer of agar.

Petri dishes 100 mm across by 20 mm deep were used to reduce air circulation and drying inside the dish. The block of agar to which the telial columns were attached was of sufficient depth that the tips of the columns were not more than 1 mm above the surface of agar in the bottom half of the dish. This closeness served the dual purpose of keeping the

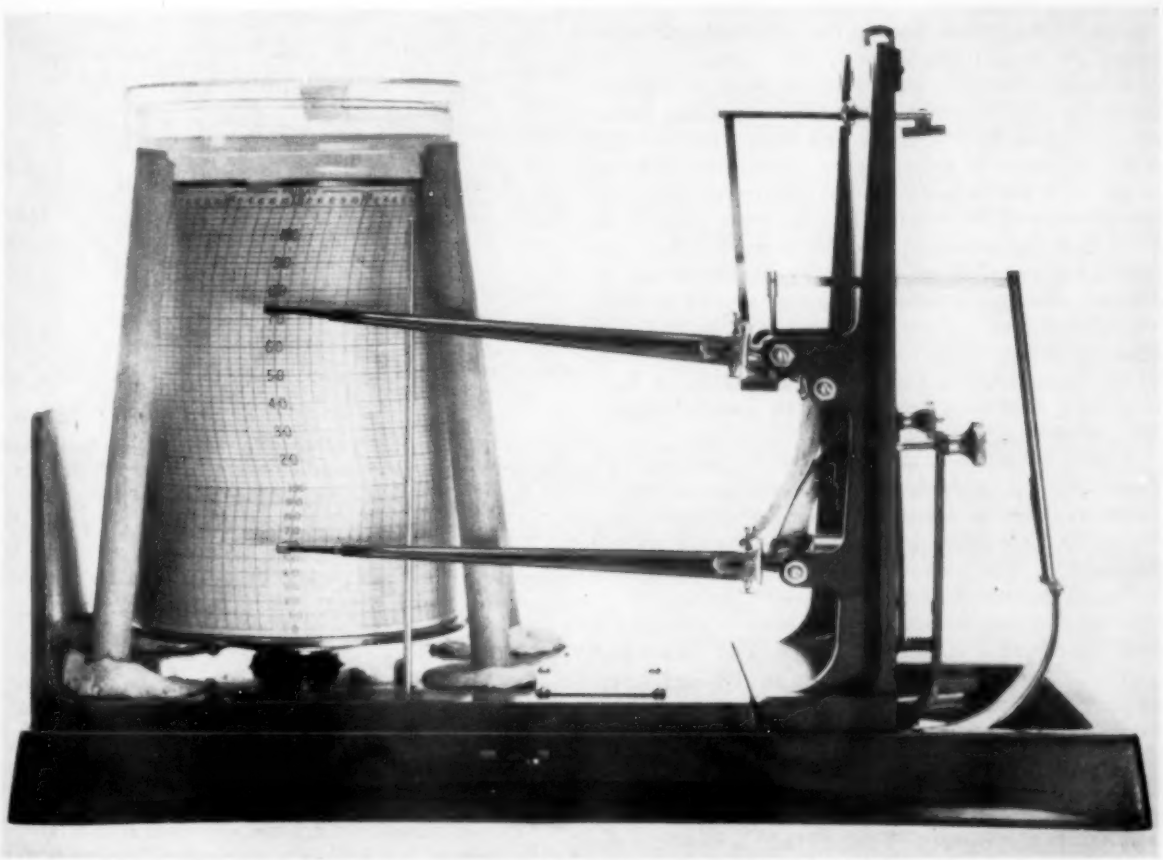

FIG. 1. Apparatus used for studying rate of production of sporidia from individual telial columns.

TABLE 1.—*The rate and capacity of sporidium production per hour from a telial column 1330μ by 105μ, age 10 days, host R. aureum kept at 16°C, germination at 15°C.*

Hours	No. of sporidia	Hours	No. of sporidia	Hours	No. of sporidia	Hours	No. of sporidia
8	[a]	24	191	40	95	56	14
9	2	25	122	41	101	57	10
10	2	26	144	42	73	58	7
11	4	27	163	43	89	59	3
12	18	28	185	44	101	60	9
13	46	29	155	45	80	61	2
14	89	30	166	46	64	62	3
15	152	31	131	47	67	63	1
16	168	32	99	48	64	64	0
17	164	33	97	49	57	65	1
18	143	34	123	50	56	66	2
19	131	35	113	51	52	67	1
20	142	36	132	52	34	68	0
21	188	37	107	53	36	69	0
22	242	38	139	54	21	70	1
23	213	39	92	55	23	Total	4,930[b]

[a] Casting started at 8.5 hours.
[b] A total of 4930 sporidia were cast during 61.5 hours. The crest of production started after 14 hours and continued through the 45th hour, for a crest totaling 31 hours.

columns at 100% humidity and minimizing the lateral distance of sporidia cast.

Both 24-hour and 7-day clocks were used, but the latter were preferred: with tests of 96–120 hours, a change of the bottom half of the dish every 24 hours risked the loss of sporidia.

To make accurate spore counts, a circle corresponding to the circumference of the clock drum was drawn on a sheet of tracing linen, and lines were radiated from the center to points on the circle corresponding to the time intervals on the hygrothermograph chart. The distance of the telial column from the edge of the Petri dish was measured, and the exact time intervals of the cast sporidia on the agar were determined as follows: the dish containing the ring of cast sporidia was placed over the tracing linen circle so that zero hours on the circle corresponded to the starting point of the experiment on the dish. The sporidia in their respective time zones could then be counted with a 30× dissecting microscope.

A second method of counting was also used in each experiment. A whipple disk in a compound microscope was used to measure the increment corresponding to the time interval as measured with the tracing linen circle. With this method the number of sporidia in each increment was counted at 100×.

EXPERIMENTAL RESULTS.—Eight tests represented a total of 22 telial columns, all formed at either 16° or 20°C. Sporidium casting began 6–11 hours (averaging 8.1) after the telia were subjected to 100% humidity on the agar blocks. The major differences noted were in total number of sporidia cast and total time involved in casting. These differences were variable and appeared to depend primarily on size and age of the column. Tables 1 and 2 illustrate the rate and capacity of sporidium production from individual telial columns of different size and age, both from the same host plant kept at 16°C.

Four tests represented 12 telial columns of various sizes and ages from *R. aureum* kept out-of-doors during the infection and telium-formation period. Daytime temperatures were 10–27°C; night temperatures were 5–14°C. Meteorological conditions were typical of spring weather at Berkeley. Periods of high fog, of sun, and of rain occurred throughout the infection period. New infections were found on ribes leaves after rain, but the rust did not spread to uninfected ribes 20 feet away.

Sporidium casting began 5.5–12.5 hours (averaging 8.8) after telia were placed on the agar blocks. As with telia formed under constant temperature, the major differences noted were in total number of sporidia cast and total time involved in casting. Again, the differences appeared to depend primarily on the size and age of the column. Table 3 illustrates the rate and capacity of sporidium production from 2 telial columns of different size and age, both from the same host plant kept out-of-doors.

In a preliminary experiment at 15°C, sporidium production in the dark was compared with production under artificial light. One chamber was kept completely dark; the other was kept at a constant 770 ft-c of illumination furnished by 19 13-watt fluorescent white-lite bulbs. Ten telia per Petri dish were used to offset any differences encountered between individual columns. After 120 hours no apparent difference was noted between columns germinating in constant light and those in constant dark. In both, sporidium casting had begun after 8 hours.

DISCUSSION.—Of particular importance in spread of the disease from ribes to white pines is what Gaumann (2) termed the "numerical threshold of infection"—the number of individuals of a parasite that is necessary to establish infection under favorable conditions. In the white pine blister rust disease, several factors must be considered: how many telium-bearing plants are present, the number, size, and viability of telia per plant, how many sporidia are

TABLE 2.—*The rate and capacity of sporidium production per hour from a telial column 378μ by 73.9μ, age 4 days; host is same plant and leaf as in Table 1, germination at 15°C*

Hours	No. of sporidia	Hours	No. of sporidia	Hours	No. of sporidia	Hours	No. of sporidia
6	[a]	15	59	24	12	33	4
7	1	16	69	25	9	34	3
8	3	17	49	26	11	35	1
9	7	18	33	27	7	36	0
10	20	19	35	28	9	37	1
11	39	20	29	29	6	38	0
12	48	21	24	30	7	39	0
13	54	22	13	31	5	40	0
14	73	23	7	32	3	Total	641[b]

[a] Casting started at 7.0 hours.
[b] A total of 641 sporidia were cast. A total crest of 11 hours is noted here.

TABLE 3.—*The rate and capacity of sporidium production per hour from 2 telial columns formed under natural conditions; host R. aureum kept out-of-doors, germination at 15°C*

Column 770μ by 73.6μ, age 7–9 days:				Column 322μ by 54.4μ, age 3–5 days:			
Hours	No. of sporidia	Hours	No. of sporidia	Hours	No. of sporidia	Hours	No. of sporidia
5	0	26	105	5	0	26	2
6	0	27	123	6	1	27	0
7	1	28	111	7	1	28	3
8	3	29	61	8	8	29	1
9	5	30	57	9	13	30	0
10	20	31	45	10	21	31	1
11	53	32	31	11	20	32	1
12	103	33	29	12	37	33	0
13	212	34	37	13	49	Total	522
14	204	35	25	14	67		
15	214	36	13	15	65		
16	191	37	10	16	55		
17	175	38	6	17	61		
18	143	39	3	18	38		
19	162	40	5	19	28		
20	123	41	1	20	15		
21	131	42	0	21	11		
22	152	43	1	22	9		
23	133	44	0	23	5		
24	145	Total	2,954	24	3		
25	121			25	7		

produced per telial column, and the duration of weather conditions optimum for maximum production and dissemination of sporidia and infection of the pine host. Many of these factors are best studied under naturally occurring conditions, and a great number of papers, too numerous to mention, have been written on field observations.

The factor of the number of sporidia produced per telial column lends itself to controlled laboratory studies. Taylor (7) estimated the number of teliospores per column by measuring the length and diameter of the column. Assuming 4 sporidia per teliospore, she determined the potential sporidium production. Although she gave no figures for the size of the column used, she reported that a representative column from *R. nigrum* had 1500 teliospores capable of producing 6000 sporidia.

In the present study the best quantitative method of measuring telial germination was based on the ability of a column to cast sporidia. Germination of a teliospore of *C. ribicola* results in the formation of a promycelium in which the diploid nucleus divides meiotically and each of the daughter nuclei is separated in the promycelium by septation. Observations have repeatedly shown, however, that 4 sporidia are not always produced from each teliospore. It is not at all uncommon for only 2 or sometimes 3 sporidia to form on a promycelium. The reason is unknown, but could be incomplete nuclear division, nuclear disintegration, or incomplete septation in the promycelium. Whatever the reason, the number of teliospores present is not an accurate method of determining the quantity of sporidia that will be produced.

Colley (1) said that teliospores average 16 by 42 μ but vary greatly in size, and that abortive undeveloped spores occasionally occur, being scattered throughout the column. Klebahn (5) gave teliospore measurements of 35–60 (average 25–56) μ long by 11–16 (average 9–14) μ thick, and said that Fischer's measurements were 70 (bis 56) μ long by 21 (bis 14) μ thick.

Because of this variation in the size and viability of teliospores in a telial column, measurements alone were not considered reliable to estimate potential sporidium production. Rather, it was considered far more accurate and realistic to measure the actual biotic capacity of a telium or its inoculum potential by counts of sporidia discharged from a column held under standard, favorable conditions.

Evidence from this study, like that from others (3, 4, 6, 9) with the same fungus, shows that relatively few hours of saturated air with low temperatures bring about germination of teliospores and subsequent casting of sporidia. The study also shows that sporidium discharge from a single telial column is not instantaneous. The rate of sporidia cast rises to maximum, continues at about the same level for a considerable period, and finally begins dropping off as the telial column approaches exhaustion. The time for complete exhaustion of the column, given favorable environmental conditions, appears to depend primarily on the size and age of the column.

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Indexing Sour Cherry Trees for Necrotic-Ring-Spot Virus on Excised Twigs.[1] HARRY C. FINK.[2] Large numbers of trees must be indexed to give confidence in virus surveys, virus-spread studies, and production of virus-free nursery stocks. Several plants—peach,[3,4] Shirofugen and Kwanzan cherry,[5] *Prunus tomentosa* Thumb.,[6] cucumber,[7] and sour cherry[8]—have been reported as reliable indicator plants for necrotic-ring-spot virus of sour cherry. The choice of researchers is often sour cherry because it is the host plant being studied. Acquisition of numbers of ring-spot-free scions and rootstocks and the time (2 years) required to produce trees of a size suitable for indicator plants are problems often difficult to resolve. Indexing on excised twigs solves these problems.

Interest in this method was aroused by the need to index large numbers of trees in southwestern Iowa nurseries. Twigs are substituted for the whole trees used in the method of Moore and Keitt,[8] which is based on "shock" symptoms during the initial stages of disease development and the latency of the virus thereafter. They demonstrated that grafting on a ring-spot-virus-infected bud produces symptoms in ring-spot-free sour cherry but not in ring-spot-infected trees. The reactions are the same when excised dormant twigs are substituted for trees.

Indexing on excised twigs was first investigated in 1949, in Iowa. Twigs were cut from virus-free scion mother trees in December, and buds containing necrotic-ring-spot virus were inserted into sections containing 6–8 buds. These budded twigs were held with their proximal ends in tap water until dormancy was broken. Symptoms occurred on the newly unfolding leaves on twigs budded with infected buds. However, growth was sometimes poor, making symptoms difficult to distinguish. In March of 1950, more twigs were obtained from the scion mother trees and the test was repeated. This time dormancy was broken in 3 weeks, growth of the leaves was enhanced, symptoms could be read easily.

To test its reliability, the excised twig method was compared with indexing on ring-spot-free sour cherry trees. Buds known to be carrying the virus were obtained through the courtesy of Dr. J. D. Moore, of the University of Wisconsin. Symptoms were expressed on leaves of both excised twigs and trees. Buds were from 19 different sources of necrotic-ring-spot virus—1 in combination with green-ring-mottle-virus, 13 in combination with sour cherry-yellows virus, 2 with recurrent ring-spot virus, and 3 with latent ring-spot virus. Twenty-five twigs from ring-spot-virus-infected trees were grafted with buds containing ring-spot virus; no symptoms resulted.

These experiments demonstrated that excised twigs could be used to index for the presence of necrotic-ring-spot virus providing the twigs had undergone a necessary dormant period.

When gibberellic acid became available, experiments were designed to determine if dormancy of cherry twigs could be broken earlier, thus extending the time available for working with this method. In January, 1958, dormant twigs were cut from Montmorency cherry trees that had been propagated from ring-spot-free root stocks and ring-spot-free scions and planted at University Park, Pennsylvania, in 1956. Each twig contained at least 6 leaf buds. Groups of 8 twigs were soaked for 5 minutes by immersion in aqueous solutions of gibberellic acid at concentrations of 100, 50, 25, 12, 6, and 3 ppm before ring-spot-infected buds were grafted to them. One lot was immersed in water before budding, 2 lots were not immersed but the proximal ends were placed in either 6 or 3 ppm gibberellic acid (instead of tap water, as were the other twigs), and all were held in the greenhouse until leaves emerged. Dormancy was broken on all gibberellic-acid-treated twigs in 3 weeks, regardless of concentration or method of application.

FIG. 1. Symptoms of necrotic-ring-spot virus on excised indicator twigs, center twig unbudded check, left and right budded with buds containing necrotic-ring-spot virus.

[1] Contribution No. 235 from the Department of Botany and Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication on May 23, 1958, as paper No. 2257 in the Journal Series.

[2] Associate Professor of Plant Pathology.

[3] Cation, D. 1942. Cherry yellows in Michigan. Mich. State Hort. Soc. Ann. Rept. 72: 115-116.

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TABLE 1.—*Results with excised twigs used as indicators for necrotic ring-spot of sour cherry*

Bud source[a]	Twig source[a]	No. of twigs budded	
		Symptoms	No symptoms
Iowa, 1949			
Mont. v.i.	Mont. v.f.	45	4
Unbudded ck.	Mont. v.f.	0	30
Mahaleb v.i.	Mont. v.f.	14	8
Mont. v.i.	E. R. v.i.	0	15
Mont. v.i.	Mont. v.i.	0	10
Pennsylvania, 1958[b]			
Mont. v.i.	Mont. v.f.	66	0
Mont. v.i.	Mont. v.f.	60	0
Unbudded ck.	Mont. v.f.	0	5

[a] Varieties Montmorency = Mont. Early Richmond = E. R. Virus free = v.f. Virus infected = v.i.
[b] Gibberellic acid used to break dormancy in all experiments in Pennsylvania.

Symptoms were expressed on all twigs grafted with buds known to contain the ring-spot virus. The experiment was repeated on March 9, 1958. Symptoms were read on March 17 on gibberellic-acid-treated twigs, and on March 26 on water-soaked twigs.

Leaf tissue showing symptoms was used to transmit the virus to cucumber seedlings (National Pickling), proving actual transmission of the virus from buds to twigs.[7] Of 345 cucumbers inoculated, 135 expressed symptoms.

Final data from all experiments are in Table 1. Symptoms on twigs are illustrated in Fig. 1.

Because only parts of large trees may contain the virus during initial stages of infection it is desirable to use several twigs excised from different portions of the tree to ensure confidence in the test. A smaller number can be used for small trees, such as nursery trees; but at least 2 should be used, regardless of size.

Effects of cross protection, of mild strains of ring-spot virus against severe ones, upon this test have not been determined. For this reason tests on twigs known to be free of the virus are more reliable than are tests on twigs excised from trees to be indexed.—Department of Botany and Plant Pathology, The Pennsylvania State University, University Park, Pa.

Some Effects of Light on the Growth and Morphology of Rhizoctonia solani.[1] RICHARD D. DURBIN.[2] Most studies on the effects of light on fungi have dealt with the reproductive rather than vegetative phase. Many authors have indicated that light is inhibitory to the vegetative growth of fungi, but little

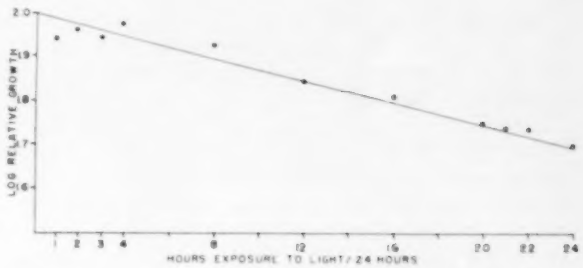

FIG. 1. Effect of daily period of illumination on the log of the mycelial dry weight of a clone of *R. solani* after 1 week's growth in shake culture.

quantitative work has apparently been done.[3, 4] This is understandable since vegetative and reproductive processes cannot be entirely separated in most fungi.

In work with clones of *Rhizoctonia solani* Kuehn (*Pellicularia filamentosa* (Pat.) Rogers) isolated from a large number of hosts,[5] it was found that, under conditions of alternating light and darkness, some clones would form sclerotia in response to light. Linear growth rates remained constant even though, superficially, growth under light appeared faster.

As part of a problem dealing with the ecology of *R. solani*,[6] this phenomenon was investigated further by determining the linear growth rate and mycelial dry weight of 9 clones under constant light or constant darkness. The light source was provided by Sylvania "cool white standard" fluorescent lights at 200 ft-c with a spectrum characterized by a sharp peak at 5800 Å and a secondary peak of 40% relative energy at 4800 Å. with a fairly rapid falling off of energy on both sides of both peaks. The cultures were grown on Czapek's medium plus 2% agar under constant conditions in a 500-ft.[3] walk-in box at 25±1°C. Linear growth rates were determined by growing the clones, replicated 8 times per treatment, in U-shaped glass growth tubes. After an initial lag, the fungus showed a constant growth rate. The test was then started and run for 5 days, growth being expressed as mm per 24 hours. Mycelial dry weights were determined by inoculating equal amounts of mycelial pellets into flasks containing liquid Czapek's medium. These were placed on a reciprocating shaker for 1 week, and then harvested and dried to a constant weight at 100°C. Twenty flasks were used for each treatment.

[1] A portion of a dissertation presented to the Graduate Division of the University of California, Berkeley, in partial fulfillment of the requirements for the Ph.D. degree. The assistance of K. F. Baker throughout this study is gratefully acknowledged.
[2] Present address: Department of Plant Pathology and Botany, Institute of Agriculture, University of Minnesota, Saint Paul.

[3] Hall, Muriel P. 1933. An analysis of the factors controlling the growth form of certain fungi, with special reference to *Sclerotinia (Monilia) fructigena*. Ann. Botany (London) 47: 543-578.
[4] Page, O. T. 1956. The influence of light and other environmental factors on mycelial growth and sclerotial production by *Botrytis squamosa*. Can. J. Botany 34: 881-890.
[5] Some of the clones used in this study were provided by L. H. Davis, B. B. Exner, F. I. Frosheiser, R. G. Grogan, E. S. Luttrell, G. F. Weber, and J. C. Wells.
[6] Durbin, R. D. Factors affecting the vertical distribution of *Rhizoctonia solani*, with special reference to CO_2 concentration. Am. J Botany (in press).

With all clones tested there were no significant differences between the 2 environmental treatments in the linear growth rates. However, with 7 of the clones, growth as evidenced by mycelial dry weight was significantly greater when the fungus was not illuminated (Table 1). When the mycelial dry weights of these clones were determined after growing 1 week in light periods of 1–24 hours per day, the log of the dry weight measurements was found to be negatively correlated with the hours of light received in a 24-hour period (Fig. 1).

These results illustrated a disadvantage of using linear measurement as a function of growth, since light duration had no effect on linear growth rate but, in most cases, significantly reduced mycelial dry weight. When Brancato and Golding,[7] using other fungi, varied the thickness of the substrate, results were comparable: linear growth rates were not affected by substrate thickness, but mycelial dry weights were.

The reason is not known why light affects mycelial dry weight but not linear growth rate. However, preliminary observations indicate that length and development of the lateral hyphae are less when the fungus is subjected to light. On the other hand, growth of the terminal hyphae appears unaffected by light. The reduced development of lateral hyphae in light may contribute to reduction of mycelial dry weight.

Gross differences were also evident in the morphology of 96 clones grown in both constant light and darkness. About a quarter of these produced sclerotia in light, but not in the dark. Another quarter produced no sclerotia under either treatment. The remaining half always produced sclerotia, but sclerotial morphology was decidedly different in most cases. In constant light the sclerotia were more appressed and compact, and occurred most in clusters nearer the center of the Petri dish. Illuminated cultures showed a distinct tendency toward less aerial mycelium, thinner surface stroma, and lighter pigmentation. The amount of pigmentation was correlated with the activity of the enzyme tyrosinase, as determined manometrically.

Since *R. solani* does not commonly produce its perfect stage in culture, even under specialized laboratory conditions, its macroscopic characteristics—such as the shape, size, color and abundance of sclerotia, amount of surface stroma and aerial mycelium, pigmentation, and growth rate—have been used to separate this heterogeneous fungus into types, forms, groups, or species. However, since these characteristics vary with environment, it is questionable whether they should be used in delimiting clones of *R. solani*.

—Department of Plant Pathology, University of California, Los Angeles 24, California.

TABLE 1.—*Effect of light on mean mycelial dry weight of 9 clones of R. solani after a week's growth in shake culture*

Host	Symptom	Illuminated (g)	Unilluminated (g)
Ficus elastica	Stem rot	0.69	0.63
Pepper	Damping-off	1.05	1.52*
Peperomia sp.	Cutting rot	0.97	1.56*
Fig	Aerial blight	0.96	1.88**
Gramineae	Aerial blight	0.68	1.91**
Iris	Rhizome rot	1.82	1.94
Azalea	Root rot	1.48	2.06*
Pepper	Root rot	1.65	2.37*
Alfalfa	Aerial blight	1.75	2.53**

* Difference exceeds the 5% level of significance.
** Difference exceeds the 1% level of significance.

[7] Brancato, F. P., and N. S. Golding. 1953. The diameter of the mold colony as a reliable measure of growth. Mycologia 45: 848-864.

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NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

Phytopathologist Richard Campana has been appointed head of the Department of Botany and Plant Pathology, University of Maine. Dr. Campana was formerly Associate Plant Pathologist on the staff of the Illinois Natural History Survey where he worked primarily in research and education on phloem necrosis and Dutch elm diseases. He received his Ph.D. in forest pathology from Yale University.

Officers of the Canadian Phytopathological Society were erroneously listed in the August number of News. The corrected listing for 1959 includes: President, M. W. Cormack; Vice-President, W. E. Sackston; Secretary-Treasurer, R. L. Millar; Councilors, H. Genereux and A. J. Skolko.

Nematode physiology will be investigated by Lorin R. Krusberg at the Rothamsted Experiment Station. Mr. Krusberg has been awarded a National Science Foundation Post-Doctoral Fellowship for the investigation. He will complete his doctorate at North Carolina State College in January and leave for England in March.

Phytopathologist R. M. Endo has accepted a position as Assistant Professor in the Department of Plant Pathology, University of California at Los Angeles. Dr. Endo was formerly plant pathologist with the Cereal Crops Branch stationed at Urbana, Illinois.

An Oak Wilt Conference organized by the U.S.D.A. Forest Service was held November 13 and 14 at West Virginia University . State and Forest Service plant pathologists considered such aspects as: Significant advances in research, present status of information on root grafts, source of inoculum and long-distance spread, and conditions for infection and eliminating infection centers. Another part of the program was devoted to considering local situations in different states, the effectiveness of oak wilt detection, post-control appraisal studies, and an objective look at control efforts.

Plant pathologist Walter Hartstirn, Oregon State College, recently joined the staff of the Ilinois Natural History Survey where he will investigate diseases of shade trees.

The Northeastern Division held its 18th Annual Meeting November 6 and 7 at West Springfield, Massachusetts. Approximately 165 people were in attendance to hear the 19 research papers and informal progress reports on results of 1958 fungicide tests. Officers elected for the coming year are: President, A. E. Rich; Vice-President, J. E. Livingston; Secretary-Treasurer, D. E. Marsden; Councilor, E. M. Stoddard.

Recent Purdue Plant Pathology graduates have accepted positions as follows: Michael P. Britton, Extension Plant Pathologist, University of Illinois; Timothy A. Gaskin, Associated Seed Growers, San Jose, California; Nade G. Vakili, United Fruit Company, Honduras.

Plant Pathologist Charles A. Schexnayder, formerly investigating sugar cane diseases at the U.S.D.A. Sugar Field Station, Houma, Louisiana, is now breeding sugar cane for disease resistance for the Central Romana Corporation, La Romana, Dominican Republic.

Aerobiological investigations are being supported at the Kansas State College by a grant of $11,500 from the U. S. Public Health Service. The research being conducted by S. M. Pady and C. L. Kramer will be concerned with hourly variation of fungus spores.

Mycologist Peter Hunt, formerly Assistant Professor of Plant Pathology at Cornell University, became Assistant Lecturer in the Department of Botany at Birkbeck College, University of London, on October 1. Dr. Hunt came to Cornell from the University of Bristol, Bristol, England, to teach courses in Mycology during the absence of Cornell Mycologist, R. P. Korf who was on sabbatic leave during the 1957-1958 academic year.

Phytopathologist N. T. Powell, a graduate of V.P.I. and North Carolina State College, was recently appointed Research Assistant Professor of Plant Pathology at North Carolina State College. Dr. Powell will do basic and applied research in the program of breeding for nematode resistance in tobacco.

After more than 40 years' service Robert W. Leukel, plant pathologist in the Cereal Crops Research Branch, U.S.D.A., retired on October 31. More than 70 friends and associates attended a farewell luncheon in his honor. Mr. Leukel is best known for his investigations on chemical control of cereal smuts and seedling diseases. He also conducted extensive investigations on diseases of sorghum.

Deaths—Royal J. Haskell, Extension Service plant pathologist from 1930 to 1957, died December 3 at the age of 68. Dr. Haskell was in charge of the Victory Garden program in World War II and had received a Superior Service Award from the U.S.D.A. He served as President of the Society in 1929.